JOURNAL OF DAIRY SCIENCE

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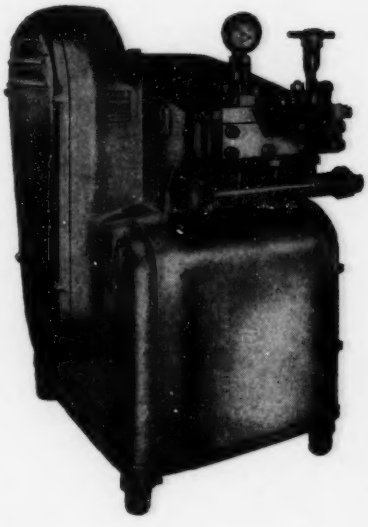

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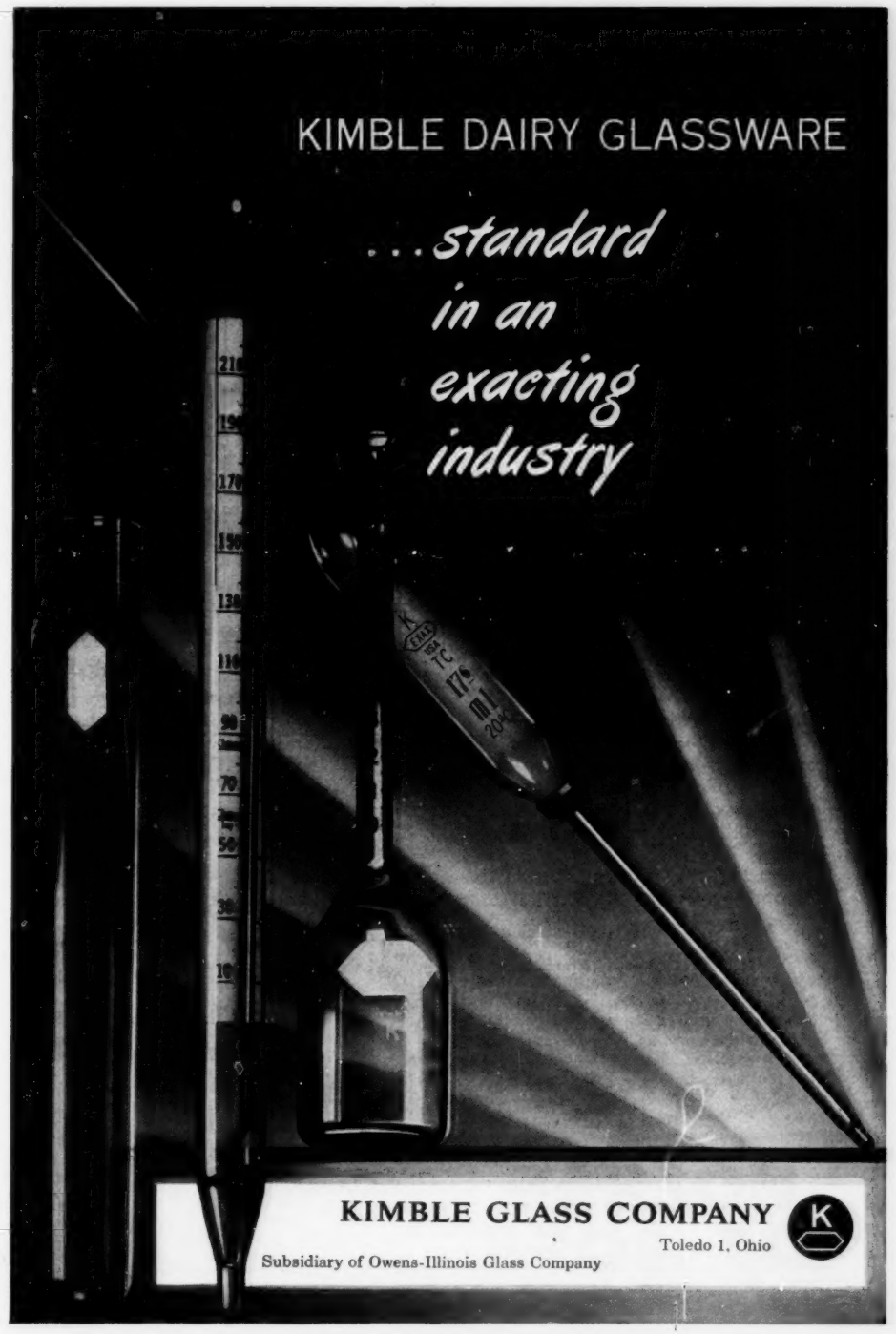

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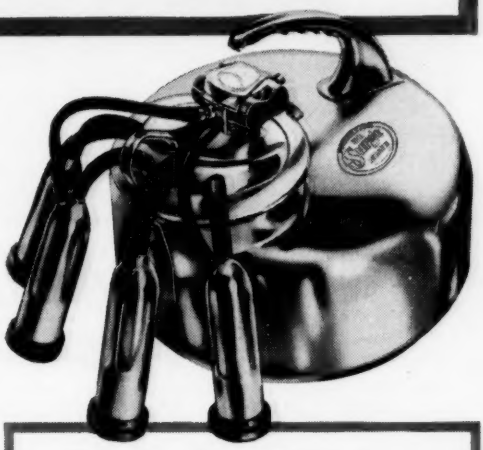

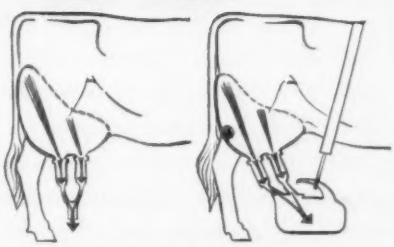

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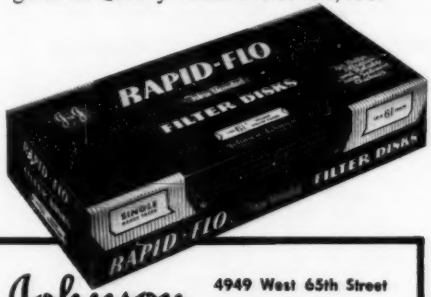

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JOURNAL OF DAIRY SCIENCE

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A PLATING MEDIUM FOR THE ISOLATION AND ENUMERATION OF ENTEROCOCCI

GEORGE W. REINBOLD, MARIANNE SWERN AND RALPH V. HUSSONG

Kraft Foods Company, Research Laboratories, Glenview, Illinois

The importance of the enterococcus group of streptococci in dairy bacteriology is widely recognized. Since the establishment of this group by Sherman (7), information gradually has accumulated concerning the occurrence of these organisms in dairy products. Sherman and Stark (8) found *Streptococcus faecalis* in Swiss cheese one day old. Tittsler *et al.* (9) determined that enterococci were one of the bacterial types most frequently isolated from ripening pasteurized milk cheese. Foster *et al.* (3) isolated enterococci from Brick cheese. Within recent years, Dahlberg and Kosikowsky (2) have been concerned with use of a special strain of *S. faecalis* as a starter for developing flavor in Cheddar cheese. Due to their appreciable heat resistance, the enterococci frequently have been found in milk powder and other dairy products normally subjected to heat treatment. The significance of their presence in these products has been assigned different values by various authorities. White and Sherman (10), after studying the occurrence of these organisms in raw milk, believe that their use as an index of milk quality is of doubtful value.

A plating medium of high selectivity for the enumération of enterococci in dairy products would be desirable. White and Sherman (10) developed a medium for this purpose. However, its use is limited by the fact that it permits the growth of lactobacilli, as reported by Kosikowsky and Dahlberg (5). Hajna and Perry (4) also formulated a medium to detect the presence of fecal streptococci. It was then shown by Ostrolenk and Hunter (6) that, although otherwise of great value, false positive and false negative reactions occurred when this medium was employed. In some instances, enterococci grew in this medium without producing the acid necessary to induce a positive reaction. The false positive reactions were due to the growth of bacilli.

EXPERIMENTAL

The fermentation of citrates by enterococci has been reported by Abd-el-Malek and Gibson (1). To attain a limited degree of selectivity, a plating medium containing 2.0 per cent sodium citrate, 1.0 per cent Trypticase, 1.0 per cent yeast extract and 1.5 per cent agar first was developed. This medium was tested against identified cultures of enterococci.

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For greater selectivity, previously sterilized aqueous solutions of ditetrazolium chloride were added to the melted basal medium. Enterococci possess the ability of converting ditetrazolium chloride, which is slightly yellowish in color in aqueous solution, to an intense blue diformazan. This color change is restricted to the colony and does not extend to the surrounding area. Since 0.001 per cent ditetrazolium chloride proved to be non-inhibitory, this level was selected for further investigation. This combination of the basal medium and ditetrazolium chloride was tested against 27 samples of raw milk. It soon became obvious that unidentified types of Gram-negative rods could give false positive reactions.

To overcome this deficiency, sterile aqueous solutions of sodium azide were incorporated in the basal medium. Concentrations of 0.01, 0.015, 0.0225 and 0.03 per cent sodium azide in the medium were tested against selected cultures of enterococci.

Twenty-four samples of raw milk were plated, using the citrate medium with 0.001 per cent ditetrazolium chloride and 0.01 and 0.03 per cent sodium azide. Representative numbers of colonies were picked into sodium citrate broth tubes. These broth cultures were examined microscopically and were stabbed into tubes of a nutritive agar containing 6.5 per cent NaCl. Growth reactions in litmus milk and 0.1 and 0.3 per cent methylene blue milk also were observed.

RESULTS AND DISCUSSION

Basal medium. The sodium citrate basal medium was tested against five different cultures of enterococci. This medium supported luxurious growth of these organisms. The number and size of colonies developing on this medium were at least comparable to those produced on tomato juice agar and B.B.L. Eugonagar. However, this medium also maintained equally good growth of other types of bacteria, including lactobacilli and the Gram-negative rods mentioned previously.

Ditetrazolium chloride. A concentration of 0.0025 per cent ditetrazolium chloride was not inhibitory to any of the five test cultures of enterococci. When the cultures were plated with the sodium citrate basal medium containing 0.0025 per cent ditetrazolium chloride, four cultures were reduced 17, 18, 19 and 12 per cent in number; the fifth culture increased 19 per cent over the negative control. Statistical analysis of variation indicated that significant inhibition had not occurred. To insure complete absence of growth retardation, a level of 0.001 per cent of the indicator was tested and selected as the desirable concentration.

Sodium azide. To determine the maximum concentration of sodium azide that could be added to the sodium citrate basal medium plus 0.001 per cent ditetrazolium chloride, concentrations of 0.01, 0.015, 0.0225 and 0.03 per cent sodium azide were tested against ten enterococcus cultures. The 0.01 per cent level had no adverse effects upon the numbers of colonies that developed on the agar plates. When 0.015 and 0.0225 per cent sodium azide was employed, the test cultures again were not affected when only the numbers of colonies that developed were considered. A slight decrease in colony size was noted. As

shown by Table 1, however, 0.03 per cent sodium azide definitely was inhibitory to five of the ten cultures of enterococci. In addition to reducing the numbers of organisms capable of growing on the poured plates of sodium citrate agar, a drastic reduction in colony size was noticed. This concentration of sodium azide, therefore, could not be used where accurate counts are desired.

Testing of the complete medium. When the sodium citrate agar plus 0.001 per cent ditetrazolium chloride was used to plate 27 samples of raw milk (Table 2), the degree of selectivity attained was low. Large numbers of Gram-negative

TABLE 1

The effect of 0.03% sodium azide upon enterococcus cultures

Culture No.	Basal medium + 0.001% ditetrazolium chloride	Basal medium + 0.001% ditetrazolium chloride + 0.03% sodium azide
1	72×10^7	30×10^7
2	64×10^7	42×10^7
3	44×10^7	52×10^7
4	72×10^7	24×10^7
5	101×10^7	68×10^7
6	276×10^7	288×10^7
7	56×10^7	11×10^7
8	65×10^7	56×10^7
9	208×10^7	294×10^7
10	67×10^7	63×10^7

Counts represent mean of duplicate plates.

TABLE 2

Percentage of types of organisms isolated from raw milk employing sodium citrate agar plus 0.001% ditetrazolium chloride as the plating medium

Milk sample	Gram-negative rods	Enterococci
	(%)	(%)
3	17	83
9	75	25
11	78	22
12	48	52
14	97	3
15	74	26
21	90	10
22	79	21
23	88	12

Eighteen samples not listed yielded only Gram-negative rods.

rods, most of which produced gas in sodium citrate broth, were recovered from each of the 27 samples. Eighteen of the 27 samples yielded only Gram-negative rods. Streptococci were found growing on only nine of the individual plating series. In addition, a lactobacillus culture was isolated from one of the milk samples.

The sodium citrate agar plus ditetrazolium chloride and two other series using 0.01 per cent and 0.03 per cent sodium azide then were tested against 24 different samples of raw milk (Table 3). The medium containing only the 0.001 per cent ditetrazolium chloride again permitted the growth of Gram-negative

TABLE 3

Numbers of organisms per ml. isolated from raw milk employing sodium citrate agar plus 0.001% ditetrazolium chloride with 0.01% and 0.03% sodium azide as the plating media

Milk sample	Basal medium plus 0.001% ditetrazolium chloride		Control medium plus 0.01% sodium azide		Control medium plus 0.03% sodium azide	
	No. Gram-neg. rods	No. enterococci	No. Gram-neg. rods	No. enterococci	No. Gram-neg. rods	No. enterococci
1	100	200	.....	.....	.....	200
2	.....	.....	.....	.....	.....	.....
3	2,000	49,000	.....	42,000	.....	31,000
4	.....	6,500	.....	3,800	.....	900
5	7,000	.....	.....	.....	.....	.....
6	.....	14,300	.....	12,400	.....	10,600
7	.....	28,900	.....	7,300	.....	8,500
8	7,600	3,000	.....	.....	.....	.....
9	.....	3,800	.....	3,100	.....	3,000
10	.....	132,000	.....	151,000	.....	80,000
11	.....	273,000	.....	38,000	.....	200
12	.....	700	.....	700	.....	500
13	.....	.....	.....	.....	.....	.....
14	100	100	.....	100	.....	.....
15	.....	.....	.....	.....	.....	.....
16	100	100	.....	200	.....	.....
17	1,200	100	.....	.....	.....	.....
18	.....	.....	.....	.....	.....	.....
19	94,000	16,000	.....	500	.....	300
20	600	300	.....	200	.....	.....
21	1,500	800	.....	600	.....	600
22	140,000	330,000	.....	192,000	.....	500
23	2,700	.....	.....	3,100	.....	2,300
	—plate broken before organisms identified.					
24	.....	100	.....	.....	.....	.....

rods. This occurred in 11 of 23 samples. Eighteen of 23 samples contained enterococci; one series of plates was accidentally broken. When 0.01 per cent sodium azide was used with the ditetrazolium chloride, the Gram-negative rods were completely inhibited. Enterococci were isolated from 15 of the 24 samples. Results indicated that if enterococci were present in the sample in appreciable numbers they would grow in the medium containing 0.01 per cent sodium azide. However, six of the counts showed a rather definite inhibition. The failure to recover enterococci from the other three samples was believed to be due to random variation in numbers normally experienced in counting bacteria by the agar plate method. With a concentration of 0.03 per cent sodium azide plus ditetrazolium chloride, only the dark blue colonies of enterococci developed. Thirteen samples were shown to contain enterococci; here inhibition, as reflected by both reduced colony size and numbers, existed.

Further investigation indicated that a level of sodium azide less than 0.01 per cent (0.005 per cent) could not be used. Large numbers of unidentified Gram-negative rods were found growing on plates containing this concentration of sodium azide.

Since 0.01 per cent sodium azide seems to be inhibitory to some cultures of enterococci, the value of this method may be reduced where exact enumeration

of enterococci is desired. Where comparative or exploratory counting of these species of organisms is required, the value of this method has been demonstrated in many instances in this laboratory.

All of the streptococci isolated through the use of 0.01 per cent sodium azide were inoculated into 0.1 and 0.3 per cent methylene blue milk and 6.5 per cent NaCl agar. Growth occurred in every instance, proving these organisms to be members of the enterococcus group of streptococci.

Preparation and use. The selective medium has the following composition:

1.0 per cent Difco yeast extract
1.0 per cent B.B.L. Trypticase
2.0 per cent sodium citrate
1.5 per cent agar

Add distilled water to volume, adjust to pH 7.0, dispense in 100 ml. amounts and autoclave at 121° C. for 20 minutes. Before using melt and cool to about 43° C.; then, add 1 ml. of a 0.1 per cent sterile aqueous solution of ditetrazolium chloride and 1 ml. of a 1.0 per cent sterile aqueous solution of sodium azide per 100 ml. of media. The ditetrazolium chloride and sodium azide solutions are sterilized by autoclaving at 121° C. for 20 minutes. Ten to 12 ml. of this medium are used per plate. After solidification, plates are stratified with a thin layer of the same medium. Plates are incubated at 37° C. for not more than 72 hours. Extended incubation will permit the growth of lactobacilli. Only blue colonies are counted.

SUMMARY

A selective plating medium for the isolation and identification of the enterococcus group of streptococci has been developed. The method is based upon the ability of the enterococci to utilize sodium citrate as an available carbon source, to convert ditetrazolium chloride to a blue diformazan and to grow in the presence of 0.01 per cent sodium azide.

Results are presented which show that the selective plating medium can be used to isolate and estimate the numbers of enterococci in raw milk.

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EFFECT OF PREVIOUS LACTOSE FEEDING UPON INTESTINAL ABSORPTION OF LACTOSE IN THE RAT [1]

JESSIE E. FISCHER [2] AND T. S. SUTTON

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It is well known that lactose may induce laxation in the rat, when moderate to large amounts of this sugar are included in the diet (5, 9). This laxation is produced because lactose is hydrolyzed in the rat small intestine at a rather slow rate (14); hence, unhydrolyzed lactose molecules remain in the lumen of the intestine for some time and exert a considerable osmotic effect, as has been shown in the dog (1, 10). Therefore, water remains in, or diffuses into, the gastro-intestinal tract and reaches the cecum and colon (5), where it continues to exert an osmotic action and to interfere with the formation of feces of normal moisture content. Thus, lactose acts in the same way as has been described for the slowly-absorbed ions of the saline catharties (7).

Adaptation to lactose diets occurs, *i.e.*, diarrhea occurs less frequently and is less severe after rats have been fed lactose-containing diets for a time (9). Several changes may be involved in this process of adaptation (8), one of which may be an increase in the rate of hydrolysis and absorption of lactose in the small intestine. In this report it will be shown that a test dose of lactose disappeared from the rat gastro-intestinal tract faster after previous feeding of a lactose-containing diet than after previous feeding of a diet containing no lactose.

EXPERIMENTAL

Twenty weanling rats in two comparable groups of ten rats each (five males and five females) were fed conditioning diets for six weeks prior to determining the absorption rate of lactose. The basal cornstarch conditioning diet fed to one group contained 67 per cent cornstarch, 24 per cent vitamin-free casein (General Biochemicals, Inc.), 5 per cent Crisco and 4 per cent salt mixture (USP XII, salt mix No. 2). This diet was adequately supplemented with vitamins (including vitamin B_{12}) and trace minerals. The lactose conditioning diet was the same as the basal cornstarch diet except that it contained 25 per cent lactose (USP, Merck) in place of an equal amount of cornstarch.

Diarrhea records were made according to the method of Riggs and Beaty (9) during the first week and during the sixth week of the feeding period. In the lactose group, diarrhea ranged from none to moderate during the first week, but had disappeared almost entirely by the sixth week; thus the rats had adapted almost completely to the 25 per cent lactose diet. The basal cornstarch diet rats showed no diarrhea in either the first or the sixth week.

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[1] Supported by a grant from the American Dry Milk Institute, Chicago, Ill.

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The procedure used for determining the absorption rate of lactose was similar to that used for galactose by Free and Leonards (6). Six rats from each group were allowed the test dose of lactose. The other four rats from each group were given *no* test dose of lactose, but were fasted, killed, and treated in the same way as will be described for the test rats, as a check on the residual reducing substances in the gastro-intestinal tract of fasted rats on the two conditioning diets. Each test rat was fasted for 24 hours and was permitted only 5 ml. of water (placed in a small dish) during the last 16 hours of this fast. After the fast, the rat was weighed. Then it was allowed to ingest from the small dish 400 mg. of lactose (98 per cent *beta*-lactose, Eastman Kodak Co.) in 25 per cent aqueous solution. Less than 4 minutes was required for the voluntary ingestion of all of the solution. After exactly one hour, the rat was decapitated, and the abdomen was opened. The stomach, small intestine and large intestine were clamped off, excised, and blended separately with their respective contents in a micro-Waring blendor. The slurries obtained were washed quantitatively into separate volumetric flasks and diluted to volumes of either 100 or 200 ml. The protein was precipitated in an aliquot by the Somogyi (13) procedure, and lactose was determined in the filtrate by the Somogyi (12) copper reduction colorimetric method, extending the heating period to 25 minutes for lactose. The Evelyn photoelectric colorimeter with filter 520 was employed. All reducing substances were calculated as lactose, although a small part of the reducing sugar remaining in the small intestine may have been galactose (4).

The amounts of lactose remaining in the small intestines were corrected for residual reducing substances. This was done by subtracting 6.7 mg., the average of the reducing substances (calculated as lactose) in the small intestines of fasted control rats given no test dose of lactose, from the observed values. No such correction was applied to the stomach segments, since an average of less than the equivalent of one mg. of lactose was found in the fasted controls. The reducing substances in the large intestine segments were about the same in fasted control rats and test rats within each dietary group, indicating that probably none of the ingested test dose of lactose reached the large intestine in one hour. Therefore, the large intestine segment was disregarded in the calculation of the absorption rate.

The amount of lactose which had disappeared from the intestine was calculated by subtracting the total lactose remaining in the stomach and small intestine from 400 mg. The difference was termed the amount of lactose "absorbed" per rat, but since there is no evidence that lactose *per se* is absorbed to any extent, the "absorption" rate value more nearly represents the lactose hydrolysis rate. The absorption coefficient (amount of sugar "absorbed" per 100 g. body weight per hour) (2) was calculated for each rat. The average results are given in Table 1. The data were treated statistically by the t test for group differences (11).

DISCUSSION

The average results (Table 1) show that the amounts of lactose left in the small intestine after one hour were about the same for both groups. The actual

amounts were 130 and 124 mg. for the lactose and starch groups, respectively. However, the average amount of lactose left in the *stomach* was significantly lower ($P < 0.01$) in the lactose-fed group, only 115 mg. as compared to 169 mg. in the starch group. The lactose group "absorbed," on the average, 155 mg./*rat*/hr., whereas the starch group "absorbed" a significantly smaller ($P < 0.01$) amount of lactose, only 107 mg./rat/hr. It is unlikely that the slower gastric emptying in the starch group resulted in the observed slower rate of lactose absorption; rather, the slower lactose absorption probably is responsible in some way for the slower emptying of the stomach in the starch group. The basis for the foregoing statement is data (8) which indicated that lactose injected into the tied-off small intestine of anesthetized rats was absorbed faster in the rats previously fed a lactose-containing diet; any influence of gastric emptying thus was eliminated.

TABLE 1

Lactose remaining in the stomach and small intestine, and lactose "absorbed" from a test dose of 400 mg. lactose ingested by rats fasted for 24 hours

Diet fed for previous 6 wks.	No. of rats	Body weight after 24-hr. fast[a]	Lactose[a] left after 1 hr. in:		Lactose[a] "absorbed"	
			Stomach	Small intestine		Absorption coefficient
		(g.)	*(mg.)*	*(mg.)*	*(mg./rat/hr.)*	*(mg./100g./hr.)*
25% Lactose	6	185	115**	130	155**	88*
		±15	±8	±15	±10	±12
Basal cornstarch	6	196	169	124	107	57
		±16	±7	±5	±7	±6

* Significantly different from basal cornstarch group ($P < 0.05$).
** Significantly different from basal cornstarch group ($P < 0.01$).
[a] Mean ± standard error of the mean.
[b] Values shown are corrected values, obtained by subtracting the average of reducing substances in the small intestine of fasted control rats from the observed values.

It is shown also in Table 1 that the lactose-fed rats absorbed significantly more ($P < 0.05$) lactose /100 g. body weight/hr. than did the starch-fed rats. The average absorption coefficients were 88 and 57 for the lactose and starch groups, respectively. Cori and Cori (3) found an absorption coefficient of 56 in the first hour after giving lactose by stomach tube. (They did not state whether the rats had been fed any lactose prior to the test of absorption, although in previous work (2) a diet containing 20 per cent "milk powder" had been fed.) Thus the average absorption coefficient of 57 for the non-lactose (basal cornstarch) group agrees with that of 56 (in the first hour) reported by Cori and Cori (3), whereas the coefficient of 88 for the lactose-conditioned group is considerably higher. Coryell and Christman (4) found the rate of lactose hydrolysis to be 67 mg./100 g. body weight/hr., with absorption of all of the glucose, but of only part of the galactose resulting from the hydrolysis, in the first hour after administering lactose by stomach tube. This hydrolysis coefficient is of about the same order of magnitude as the "absorption" coefficients in the present report and in the report of Cori and Cori (3).

The faster "absorption" of lactose from the small intestine of rats previously fed the lactose diet probably is one important factor in adaptation to the lactose diet.

SUMMARY

The rate of intestinal "absorption" of lactose in the rat was greater after previous feeding for six weeks of a diet containing 25 per cent of lactose than after similar feeding of a comparable diet containing no lactose. The mean "absorption" coefficients (± the standard error of the mean) were 88 ± 12 and 57 ± 6 mg./100 g./hr., for the 25 per cent lactose-fed and the non-lactose fed groups, respectively.

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SOME SIMILARITIES OF MONOZYGOTIC CATTLE NOSEPRINTS[1]

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Although such similarities as height, weight, spots, location of hair whorls, length of tail and general appearance have all been considered as indications of monozygosity, no criterion has been formulated to positively establish identity. Gilmore (1) has suggested that noseprints from identical animals are similar; therefore, this characteristic may be used as an indication of monozygosity. Since Petersen (3) has established that noseprints may be used as positive proof of identification, Hirsch *et al.* (2) have formulated a classification for rapid identification of the print. This classification is based on certain muzzle configurations resulting from rows of sweat glands. Inasmuch as the general characteristics are similar, it seems reasonable to assume that certain similar muzzle configurations might be an indication of monozygosity. This paper deals with some of the classified similarities and differences between animals considered monozygotic.

RESULTS AND DISCUSSION

Thirty-one pairs of twins and one set of triplets, considered identical as to height, weight, hair whorls, production and general appearance, have been noseprinted and classified according to the methods previously described (2). As shown by Table 1, 25 of the 31 pairs, or 80.6 per cent, have the same general pattern. Of these 25 pairs, 19 (76 per cent) have one or more secondary like characteristics. The four pairs with a general *A* pattern have one other common secondary characteristic. Of the nine pairs having a common *B* pattern, eight of these pairs have two similar secondary characteristics. One pair had a single secondary characteristic. Of the six pairs having a general *C* pattern, each had one similar secondary characteristic. The one set of identical triplets noseprinted had a similar general pattern in the *C* group and one similar secondary characteristic. One pair of animals has been classified with a general *D* pattern and also found to have one secondary segment. In addition, it is well to mention that the *D* pattern is far less frequently observed than any of the others. Of the seven pairs having dissimilar general patterns only three have any single secondary segment of the print the same. Thus, even though there are enough individual differences between noseprints to positively establish identity, these data suggest the possibility of using noseprints as an added criterion for establishing monozygosity.

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TABLE 1

Classification of monozygotic bovine noseprints

Animal no.	Breed	Sex	Pattern	Type of ridges
T1[a]	Guernsey	Female	B	3/2 1/3 2/3
T2	Guernsey	Female	B	3/3 3/3 2/2
T3	Jersey	Female	C	2/2 2/2 2/2
T4	Jersey	Female	C	2/2 1/1 3/3
T7	Guernsey	Female	B	2/2 2/3 3/2
T8	Guernsey	Female	B	1/1 1/1 2/2
T9	Holstein	Female	B	1/1 3/1 2/2
T10	Holstein	Female	B	1/1 1/3 2/2
T11	Holstein	Female	A	3/3 3/3 2/2
T12	Holstein	Female	B	3/2 1/3 2/3
T18	Holstein	Female	B	3/3 3/3 2/2
T19	Holstein	Female	B	3/3 2/3 2/2
T34	Holstein	Female	B	2/2 1/3 2/2
T35	Holstein	Female	B	2/2 3/3 3/2
T41	Holstein	Female	B	1/1 1/3 2/2
T42	Holstein	Female	B	1/1 1/1 2/2
T45	Holstein	Female	A	1/3 1/2 2/3
T46	Holstein	Female	B	3/1 1/1 2/2
T47	Guernsey	Male	C	1/2 1/3 2/2
T48	Guernsey	Male	C	3/3 3/2 2/2
T49	Guernsey	Female	A	1/3 1/3 2/2
T50	Guernsey	Female	C	2/2 2/2 2/2
T51	Guernsey	Female	B	1/2 1/1 2/3
T52	Guernsey	Female	A	2/2 1/2 3/3
T53	Guernsey	Female	C	1/2 2/2 3/1
T54	Guernsey	Female	C	1/2 2/2 3/3
T57	Guernsey	Female	B	3/3 1/3 2/3
T58	Guernsey	Female	B	2/2 3/2 2/2
T59	Guernsey	Female	B	2/2 1/3 1/3
T60	Guernsey	Female	B	2/2 3/2 3/2
T61	Guernsey	Female	A	3/2 1/2 3/3
T62	Guernsey	Female	A	3/3 2/1 3/3
T63	Milk Short.	Female	C	3/3 3/3 3/3
T64	Milk Short.	Female	C	2/1 2/1 3/2
T67	Guernsey	Female	B	2/2 1/2 3/3
T68	Guernsey	Female	B	2/2 3/3 3/2
T69	Holstein	Female	B	2/2 3/3 2/2
T70	Holstein	Female	B	2/2 2/3 2/2
T75	Guernsey	Male	C	1/3 1/3 2/2
T76	Guernsey	Male	C	2/3 3/3 2/2
T77	Holstein	Female	B	2/2 2/3 3/2
T78	Holstein	Female	B	1/1 1/1 2/2
T79	Milk Short.	Female	B	2/2 3/3 2/2
T80	Milk Short.	Female	B	2/2 2/1 2/2
T84	Milk Short.	Female	B	1/1 3/2 2/2
T85	Milk Short.	Female	A	3/3 2/3 3/2
T88	Holstein	Female	A	2/3 2/2 3/3
T89	Holstein	Female	A	2/2 1/1 3/3
T98	Jersey	Female	C	2/2 2/2 2/2
T99	Jersey	Female	D	2/2 1/1 2/2

TABLE 1—Continued

Classification of monozygotic bovine noseprints

Animal no.	Breed	Sex	Pattern	Type of ridges
T100	Milk Short.	Female	A	2/1 2/1 2/2
T101	Milk Short.	Female	A	1/1 2/2 2/2
Tr24	Milk Short.	Female	C	2/2 2/3 2/2
Tr25	Milk Short.	Female	C	3/2 1/3 2/2
Tr26	Milk Short.	Female	C	1/3 3/3 2/2
Tw5	Milk Short.	Female	B	3/1 3/1 2/2
Tw6	Milk Short.	Female	B	2/2 3/1 2/2
Tw7	Brown Swiss	Male	D	2/2 2/2 1/1
Tw8	Brown Swiss	Male	D	2/2 3/3 3/3
Tw9	Holstein	Female	B	1/3 1/3 2/2
Tw10	Holstein	Female	D	3/3 2/2 3/3
Tw11	Holstein	Female	C	1/1 1/1 2/2
Tw12	Holstein	Female	C	3/3 1/2 2/2
Tw13	Brown Swiss	Female	A	3/1 2/2 2/2
Tw14	Brown Swiss	Female	A	1/3 3/3 2/2

[a] T = Twin from U. of Minnesota. TR = Triplet from U. of Minnesota. Tw = Twins of S. D. State College.

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THE EFFECT OF EXPOSURE TO HIGH AMBIENT TEMPERATURE ON SPERMATOGENESIS IN THE DAIRY BULL[1]

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The effects of seasonal influences on reproductive activity in domestic animals have been reviewed in detail by Anderson (4), and seasonal variations in reproductive activity in the dairy bull have been reported by several investigators (1, 2, 3, 5, 7, 12). Recently, Weeth and Herman (13) have reported a seasonal variation in the motility and longevity of dairy bull semen, indicating a mid-summer maximum followed by a sharp decline in September and then a significant increase in October, suggesting a delayed detrimental effect of high environmental temperature. Mercier and Salisbury (8) reported a low level of sperm production during June and July in five Holstein and five Guernsey bulls, accompanied by lower than average levels of fertility for these 2 months. Subsequent studies by Mercier and Salisbury (9, 10) indicate that hours of daylight are a predominant factor in seasonal variations in fertility. Erb and Waldo (6) observed a relationship between season and non-return rates in bulls used for artificial breeding. The rates were lowest in January, February, March and April and gradually increased to the highest level in September, October and November.

This study was initiated in conjunction with investigations of heat tolerance to determine some of the basic factors involved in seasonal variations in reproductive activity and to determine the effects of high environmental temperatures on spermatogenesis in the dairy bull. This report represents the progress of these investigations to date.

EXPERIMENTAL

Maintenance conditions. This study was conducted using two experimental chambers, A and B, equipped to handle two animals each. These chambers were insulated rooms in which temperature and humidity could be controlled and maintained at specified conditions over extended periods. Eight 100-watt daylight fluorescent bulbs were installed in each chamber. These lights were on from approximately six to six each day throughout the experiment.

Four Guernsey bulls were used. Bulls A-1 and A-2 were assigned to chamber A; bulls B-1 and B-2 were assigned to chamber B. A-1 and B-1 were obtained from private herds in western North Carolina and were 34 and 28 months old, respectively. A-2 and B-2 were from the College herd and were 24 and 19 months old, respectively. The bulls were tied in individual stalls within the chambers. They were fed hay and a grain mix twice daily and had free access to water from individual drinking cups.

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The original design of the experiment was to subject the animals in chamber A to slowly increasing temperatures and those in chamber B to slowly decreasing temperatures. Then the procedure was to be reversed by subjecting the bulls in chamber B to increasing temperatures and those in chamber A to decreasing temperatures. However, the water supply failed, resulting in a lack of refrigeration during the period when chamber B was being maintained at approximately 50° F. This necessitated raising the temperature in chamber B to approximately 85° F., the minimum temperature which could be maintained.

All bulls were subjected to an initial dry bulb temperature of 70° F. and a dew point of 55 to 56° F., corresponding to a relative humidity of 60 per cent. These conditions were chosen arbitrarily as starting points from which the temperature and dew point could be raised and lowered over approximately equal ranges. As the temperature in each chamber was changed, the dew point was adjusted accordingly to maintain the same vapor pressure differential between the dry bulb and the dew point temperatures (Table 1) in an attempt to keep

TABLE 1

Environmental chamber conditions to which the bulls were exposed

Chamber	Duration of exposure	Av. dry bulb temp.	Av. dew point temp.	Av. absolute humidity	Av. relative humidity	Air flow range	Vapor pressure differential
	(d.)	*(° F.)*	*(° F.)*	*(gr./lb. dry air)*	*(%)*	*(ft./min.)*	*(in. Hg.)*
A	27	70.7	54.7	64.2	58.6	.....[a]	.3262
	21	80.3	67.9	103.5	66.2	20-35	.3527
	36	89.6	79.9	155.2	73.5	15-55	.3753
	17	98.9	92.5	277.6	81.4	15-50	.3339
	42	69.3	56.5	69.7	64.4	15-60	.2617[b]
B	27	70.2	53.8	62.0	58.1	20-60	.3349
	21	58.7	41.0	38.2	52.6	15-30	.2405[b]
	17	52.2	41.0	39.6	67.5	15-80	.1282[b]
	36	86.4	77.2	141.1	73.1	10-40	.3277
	42	71.3	56.1	68.6	58.9	15-55	.3192

[a] Data not available.

[b] Low vapor pressure differential due to limitations of the air conditioning system. Dew point could not be reduced to desired level.

the loss of body heat by evaporation constant. The bulls were subjected to continuous exposure at the various temperatures except at 100° F., at which the exposure was from six to six. It was felt that 12 hours of exposure at 100° F. would provide the maximum stress under which survival for an extended period would be possible. In addition, the bulls were subjected to each set of chamber conditions over a period long enough to note any reactions and to determine if and when physiological adjustment occurred. This usually required from two to three weeks.

At the termination of the chamber exposures, the bulls were subjected to field exposure. The first period of field exposure was from September 13 to October 9, 1951, inclusive, a period of 27 days, at the end of which time bulls A-2 and B-2

were slaughtered. Bulls A-1 and B-1 continued under field conditions for a second period of 33 days, from October 10 to December 11, 1951, inclusive.

Collection of data. Dry bulb and dew point temperatures in the chambers were continuously recorded by means of Foxboro instruments,[2] and daily averages were determined from the recordings at 9, 3, 9 and 3. Since conditions were relatively constant, the average of the four readings was considered to represent a mean for the 24-hour period.

Air flow readings (feet per minute) were taken in each chamber at 24-hour intervals by means of a hot wire anemometer.[3] Temperatures and air movements during the periods of field exposure were determined from data supplied by the U. S. Weather Bureau Station at State College.

For the study of spermatogenic activity, semen samples were collected at five-day intervals by means of an artificial vagina. The bulls were removed from the chambers for collection and returned to the chambers immediately following. In general, the time required for collection was less than 15 minutes for each animal. The total volume of ejaculate and estimates of motility were determined immediately following collection. The concentration of spermatozoa was determined by haemocytometer counts. During the latter part of the study, a portion of each ejaculate was stained, using the method of Shaffer and Almquist (11), and counts were made for living, dead and abnormal sperm. Two counts of 100 sperm cells each were made on each sample, and the average of the two was used to represent the percentage of the various categories.

RESULTS

General observations. With increasing chamber temperatures, stress was more pronounced in bulls A-1 and A-2, than in B-1 and B-2. Stress, which was not obvious until the ambient temperature exceeded 90° F., was manifested by restlessness, mouth breathing accompanied by rapid, shallow respiratory movements and decreased food and water consumption. During later stages at approximately 100° F., the animals became lethargic, arose with difficulty and stood with lowered heads. Bull A-2, especially, showed severe symptoms of stress. By the end of each 12 hours of exposure to approximately 100° F. this animal was down and could be made to arise only with much difficulty and after much effort on his part. He showed marked increases in respiration, body temperature and salivation, and exhibited severe tremors and ataxia in the hind quarters. In the opinion of the investigators this animal could not have survived continuous exposure at 100° F. for 24 hours. The tremors and ataxia became continuous during the latter stages of this experimental period and persisted after the animal was turned out to the pasture lot. At the time of slaughter, 69 days following chamber exposure at 100° F., slight tremors still were present, but symptoms of ataxia had disappeared.

During the period of exposure to highest ambient temperature, all four animals showed losses in weight ranging from 0.3 to 5.6 pounds per day, weakness

[2] Manufactured by the Foxboro Co., Foxboro, Mass.

[3] Alnor Velometer, manufactured by the Illinois Testing Laboratories, Inc., Chicago, Ill.

and some loss of libido. The bulls in chamber A became slow in mounting for collection, A-2 primarily because of his weakness and ataxia, but never failed to mount and thrust with vigor. The bulls in chamber B, not being exposed to as high ambient temperature as those in chamber A, did not exhibit any additional symptoms of stress, such as were reported above.

Semen studies. The volume of ejaculate of the bulls in chamber A (Table 2) was neither markedly nor consistently affected by increases in ambient temperature. In chamber B the volume of ejaculates of both bulls increased as the ambient temperature was reduced, then decreased during the subsequent period of increased temperature at 86.4° F. Upon return to approximately 70° F. and under field conditions, the ejaculate volumes remained above pre-stress levels for B-1 and decreased to pre-stress levels for B-2.

TABLE 2

Summary of semen motility, volume and concentration of ejaculates from bulls exposed to varying environmental conditions

Bull	Chamber conditions		No. of ejaculates	Av. vol.	Av. sperm concentration	Av. total sperm	Av. initial motility
	Dry bulb	Dew point					
	(°F.)	*(°F.)*	*(5-d. intervals)*	*(ml.)*	*(1000/mm³)*	*(millions)*	*(%)*
A-1	70.7	54.7	5	3.2 ± 0.7[a]	1128 ± 147	3610 ± 965	44.0 ± 6.1
	80.3	69.7	4	2.4 ± 0.8	945 ± 164	2268 ± 1012	42.5 ± 6.8
	89.6	79.9	7	4.4 ± 0.6	841 ± 124	3700 ± 765	31.4 ± 5.2
	98.9	92.5	4	2.6 ± 0.8	1058 ± 164	2750 ± 1012	20.0 ± 6.8
	69.3	56.5	8	3.6 ± 0.6	179 ± 109[b]	644 ± 590	25.0 ± 4.8
	71.6[c]	58.6	4	4.9 ± 0.8	524 ± 104	2568 ± 835	37.5 ± 6.8
	54.3[d]	42.5	2	2.8 ± 1.1	380 ± 217	1064 ± 1181	45.0 ± 9.6
A-2	70.7	54.7	4[e]	2.4 ± 0.7	1223 ± 244	2935 ± 1625	60.0 ± 8.6
	80.3	67.9	4	3.9 ± 0.7	1275 ± 244	4972 ± 1625	52.5 ± 8.6
	89.6	79.9	7	2.5 ± 0.5	1034 ± 184	2585 ± 1229	38.6 ± 6.5
	98.9	92.5	4	4.7 ± 0.7	939 ± 244	441 ± 1625	22.5 ± 8.6
	69.3	56.5	7[f]	2.5 ± 0.5	29 ± 10	72 ± 25	1.4 ± 6.2
	71.6[c]	58.6	4	2.0 ± 0.7	3 ± 13	6 ± 33	2.5 ± 8.2
B-1	70.2	53.8	5	2.8 ± 0.7	916 ± 131	2564 ± 717	64.0 ± 9.0
	58.7	41.0	4	4.1 ± 0.8	675 ± 146	2768 ± 802	55.0 ± 10.1
	52.2	41.7	3	5.9 ± 1.0	710 ± 169	4189 ± 926	50.0 ± 11.6
	86.4	77.2	8	4.2 ± 0.6	752 ± 103	3158 ± 567	43.8 ± 7.1
	71.3	56.1	8	4.7 ± 0.6	230 ± 76[b]	1081 ± 389	17.5 ± 7.1
	71.6[c]	58.6	4	3.1 ± 0.8	96 ± 108	298 ± 550	55.0 ± 10.1
	54.3[d]	42.5	2	4.8 ± 1.2	458 ± 153	2198 ± 778	50.0 ± 14.2
B-2	70.2	53.8	5	2.7 ± 0.8	794 ± 143	2144 ± 969	52.0 ± 8.8
	58.7	41.0	4	4.0 ± 0.9[g]	433 ± 160	1928 ± 1083[g]	42.5 ± 9.9
	52.2	41.7	3	5.6 ± 1.0	608 ± 185	3404 ± 1250	40.0 ± 11.4
	86.4	77.2	8	4.9 ± 0.6	597 ± 113	2925 ± 766	21.3 ± 7.0
	71.3	56.1	8	2.3 ± 0.6	20 ± 7	46 ± 15	0.0 ± 0.0
	71.6[c]	58.6	4	2.8 ± 0.9	9 ± 10	25 ± 21	0.0 ± 0.0

[a] ± values following means represent std. errors computed on a within bull basis.
[b] Average of 7 ejaculates.
[c] First field period.
[d] Second field period.
[e] Only 4 ejaculates since bull A-2 failed to serve once during this period.
[f] Only 7 ejaculates since one was too thin to evaluate or count.
[g] Average of 3 ejaculates.

The average initial motility, sperm concentration and total sperm count decreased for all animals either during or following exposure to high ambient temperature (Table 2). In chamber A, under increasing ambient temperature, the sperm of A-1 showed steadily decreasing initial motility with each period of increased temperature then returned to the original level with reduced ambient temperature. However, for A-2 the initial motility continued to decrease following exposure to high temperature and was rated zero for the last several ejaculates collected under lowered temperature and field conditions. The same general picture was true in chamber B. There the initial motilities showed a slight decrease under reduced ambient temperature, with a marked drop occurring following the high temperature period with respect to B-1 and during the high temperature period for B-2. The semen of B-1 regained its initial motility rating under field conditions, whereas that of B-2 continued to decline and showed no motility up to the time of slaughter.

Sperm concentration and total counts were not affected appreciably until after periods of maximum temperature in both chambers, when a marked reduction occurred after exposure to an average of 98.9° F. in chamber A and 86.4° F. in chamber B. Following chamber exposure, while under field conditions, some recovery was evident in A-1 and B-1, whereas A-2 and B-2 showed a progressive decrease of spermatogenesis.

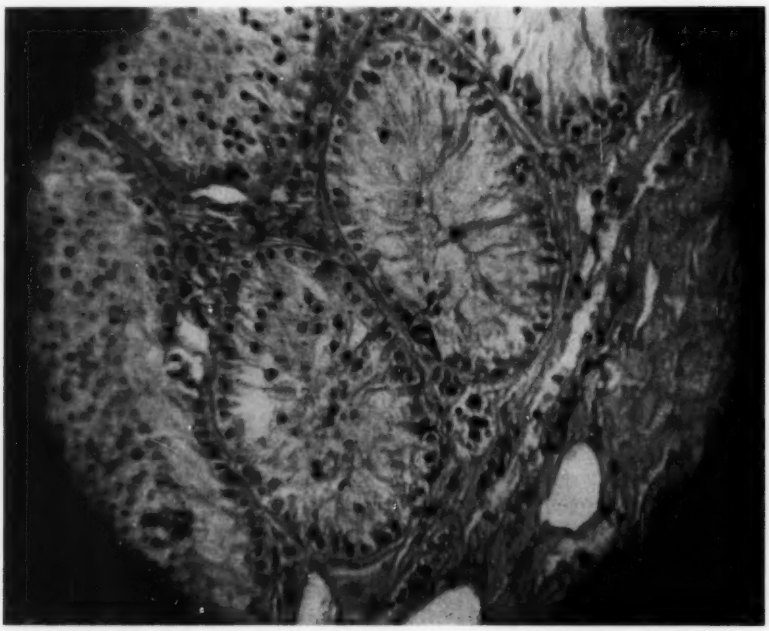

FIG. 1. Section of bull testis (A-2) indicating degeneration of germinal epithelium and lack of spermatogenesis following exposure to an average ambient temperature of 98.9° F. for approximately two weeks. (× 400).

Histological sections of the testes were prepared from each of the four bulls. Tissue for sectioning was obtained from A-2 and B-2 immediately following slaughter, at the termination of the first period of field exposure. Tissue samples from A-1 and B-1 were obtained by needle biopsy during the second period of field exposure. Figures 1 and 2 illustrate typical differences observed between the testicular tissue of the bulls which showed no signs of recovery and those which did. Note the lack of development of the germinal epithelium, lack of mitotic figures and apparent sertoli cell syncitium in the seminiferous tubules of A-2 where spermatogenesis was affected most profoundly. In contrast, spermatogenesis is evident in A-1, as indicated by mitotic figures and mature spermatozoa. The arrow in Figure 2 points out one of several cells in the section undergoing mitosis.

Table 3 shows the changes as observed under a differential staining technique. As previously mentioned, staining was not begun until the period of maximum temperature; therefore, data are available only for that and subsequent periods. Following exposure at 98.9°F. in chamber A, under lowered chamber temperature, the semen of A-1 showed an increased percentage of living and normal sperm and a decrease in total abnormal sperm. These changes continued under field conditions, indicating recovery and increased spermatogenesis following the detrimental effects suffered under high ambient temperatures. On the other

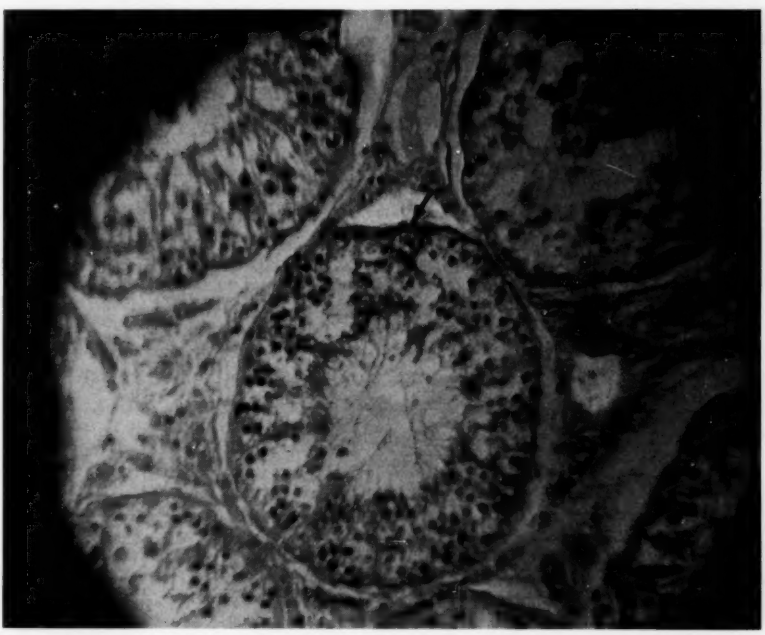

FIG. 2. Section of bull testis (A-1), indicating partial recovery of spermatogenesis following degeneration after exposure to an average ambient temperature of 98.9° F., 12 hours per day, for approximately two weeks. (× 400).

TABLE 3

Evaluation of sperm abnormalties in ejaculates of bulls exposed to varying environmental conditions

Bull	Chamber conditions		No. of ejaculates	Initial motility	Stained (dead)	Unstained (living)	Normal	Coiled tails	Tail-less	Abnormal head	Abnormal body	Total abnormal
	Dry bulb	Dew point										
	(°F.)	(°F.)	(5-d. intervals)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
A-1	98.9	92.5	4	20.0	74.1	25.9	61.8	22.9	12.9	0.9	1.5	38.2
	69.3	56.5	8	25.0	69.2	30.8	69.3	13.3	9.7	5.0	2.7	30.7
	71.6[a]	58.6	4	37.5	65.4	34.6	83.9	8.4	4.9	1.9	0.9	16.1
	54.3[b]	42.5	2	45.0	38.0	62.0	95.5	2.5	1.5	0.3	0.2	4.5
A-2	98.9	92.5	4	22.5	76.8	23.2	71.1	17.4	9.1	1.9	0.5	28.9
	69.3	56.5	7[c]	1.4	99.3	0.7	70.0	14.5	10.9	3.8	0.8	30.0
	71.6[a]	58.6	4	2.5	Unable to stain							
B-1	86.4	77.2	4	35.0	66.1	33.9	83.9	10.0	1.9	3.8	0.4	16.1
	71.3	56.1	8	17.5	79.2	20.8	64.7	16.6	12.8	4.2	1.7	35.3
	71.6[a]	58.6	4	55.0	58.5	41.5	81.9	10.2	5.1	1.5	1.3	18.1
	54.3[b]	42.5	2	50.0	37.0	63.0	97.0	1.2	1.0	0.8	0.0	3.0
B-2	86.4	77.2	4	2.5	97.0	3.0	47.6	22.8	24.1	4.9	0.6	52.4
	71.3	56.1	4[d]	0.0	100.0	0.0	66.9	17.1	4.6	9.0	2.4	33.1
	71.6[a]	58.6	4	0.0	Unable to stain							

[a] 1st field period.
[b] 2nd field period.
[c] 1 ejaculate was too thin to stain and evaluate.
[d] 4 ejaculates were too thin to stain and evaluate.

hand, A-2 did not show this partial recovery with reduced chamber temperature or under field conditions.

Following exposure to an average temperature of 86.4° F., B-1 showed a temporary decrease in per cent living and normal sperm but recovered during the subsequent period under field conditions. Here the decrease in initial motility, concentration and total count indicated in Table 2, under lowered temperature and field conditions, is directly reflected in the staining results of living and abnormal sperm. Decreased spermatogenesis in B-2 clearly is indicated by complete sperm mortality, even though the per cent of sperm showing normal morphology was increasing under field conditions. As with A-2, the last few ejaculates from B-2 were so low in sperm concentration as to prohibit proper staining and examination.

DISCUSSION

Inhibited spermatogenesis was evident in all four bulls exposed to elevated ambient temperatures. All indices used to determine sperm normality and viability showed marked changes toward a lowered reproductive capacity. A similar effect was produced under two different sets of conditions involving temperature and duration of exposure. Possibly the semen of A-1 and A-2 might have exhibited impaired spermatogenesis if these bulls had been subjected to an average temperature of 89.6° F. for 36 days, comparable to the conditions which produced the effects in chamber B. This question should be subjected to closer investigation during subsequent trials.

The data suggest that continuous exposure to environmental temperatures exceeding 85° F. may have a marked detrimental effect on spermatogenesis. The permanence of such effects can be only estimated at present, but it would seem that the "sterility" produced is of a temporary nature. Although A-2 and B-2 failed to show recovery of spermatogenesis in the ejaculated semen, Figure 1 indicates that germinal epithelium is present. This fact suggests that the time allowed for possible recovery may have been too short. If such is the case, it emphasizes that a considerable period is required for changes to appear in the ejaculated semen.

Age differences also may be of significance in influencing the degree of spermatogenic impairment. Bulls A-1 and B-1, which maintained higher quality semen during and after exposure to high temperatures, were 34 and 28 months old, respectively. Bulls A-2 and B-2 were 24 and 19 months old, respectively.

The two older and seemingly less affected bulls were raised under different conditions than were A-2 and B-2. The latter two were raised under close confinement at the State College Dairy Farm, while A-1 and B-1 were raised under typical field conditions, spending a great deal of their early life exposed to the elements and forced to forage for themselves. This difference in management could be reflected in the ability of A-1 and B-1 to better withstand environmental stress.

The results indicate that the duration of exposure is important and that subjection to 85 to 90° F. for approximately five weeks may interfere with sperma-

togenesis, sperm viability and sperm normality to an extent similar to exposure to approximately 100° F. for two weeks. In view of these observations, the data indicate that the critical temperature for spermatogenesis in the young dairy bull is between 80 and 90° F. under conditions of continuous exposure.

That the neuro-muscular nervous system may be involved in heat stress is suggested by the reactions of bull A-2, subjected to 99° F., in which tremors of the hindquarters and generalized ataxia occurred, causing him to walk with a rolling staggering gait. That this was not a permanent impairment was shown by the fact that almost complete recovery had occurred by the time the animal was slaughtered 10 weeks later.

Results of chamber trials cannot be applied to field conditions, and vice versa. The environmental chamber offers the advantage of studies under controlled conditions and is a valuable research tool. However, the results of chamber studies must be evaluated separately until sufficient field investigations are completed for correlation with those of the chamber.

SUMMARY

A preliminary report is presented, involving four young Guernsey bulls, varying in age from 19 to 34 months. These bulls were subjected to continuous exposure at various ambient temperatures in two experimental chambers. Temperatures ranged from 70 to 99° F. in one chamber and from 70 to 52° F. and 52 to 86° F. in the other chamber.

Stress, manifested by restlessness followed by lethargy, excess salivation, generalized weakness, hanging of the head when standing and tremors and ataxia in one animal, was not apparent until the temperature approached or exceeded 90° F.

Ejaculate volume was not appreciably affected, although average initial motility, sperm concentration and total sperm counts decreased markedly for all animals either during or following exposure to high ambient temperature. Impaired spermatogenesis occurred following exposure to approximately 100° F. for two weeks in two animals and approximately 86° F. for five weeks in the other two. Two bulls showed recovery of impaired spermatogenesis, but the other two bulls produced ejaculates practically void of sperm for as long as two months following chamber exposure. Sex drive was not seriously impaired in any of the four bulls.

The two bulls most seriously affected by extremes of environmental temperature were the younger of the four and were raised under management conditions quite different from the bulls which seemed to possess the most tolerance.

It is concluded that, under chamber conditions, spermatogenesis in the young dairy bull may be impaired when the animal is continuously exposed to temperatures exceeding 85° F. for periods exceeding five weeks. Further investigations are in progress to determine more precisely the optimum and critical temperatures for spermatogenesis and fertility in the dairy bull.

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FURTHER STUDIES ON THE FOLIC ACID AND VITAMIN B_{12} CONTENT OF COW'S MILK[1]

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The folic acid and vitamin B_{12} content of cow's milk has been reported by Collins, Harper, Schreiber and Elvehjem (4). The colostrum from one Guernsey cow was observed to contain higher levels of these vitamins than the milk; therefore, further analyses have been made in an effort to determine the average folic acid and vitamin B_{12} content of the colostrum of the various breeds of dairy cows. Since the data reported by Collins *et al.* (4) represent samples obtained only during the summer of 1950, the content of these vitamins in cow's milk during other seasons also was investigated.

EXPERIMENTAL AND RESULTS

The folic acid and vitamin B_{12} content of the milk samples was determined microbiologically using the methods described by Collins *et al.* (4). The vitamin B_{12} activity was measured using *Lactobacillus leichmannii* ATCC (4797), and the folic acid activity was measured using *Streptococcus faecalis* R. The free vitamin activity was determined acidimetrically by adding the diluted whole milk samples directly to the assay tubes. A 100 per cent recovery was observed when 3 γ of vitamin B_{12} were added to 1 liter of cow's milk. The vitamin B_{12} activity of cow's milk was found to be destroyed by autoclaving at pH 12 for 30 min. Thus, alkali-stable desoxyribosides do not appear to be present in cow's milk at concentrations which would influence the analysis of vitamin B_{12}.

Previous work (4) has shown that rapid microbiological synthesis of folic acid acitivity may result if samples are not analyzed within several days following collection, even when maintained at 4° C. after the addition of toluene. For this reason the milk samples were frozen immediately in order to prevent bacterial activity.

The vitamin content of colostrum and milk of the Holstein, Guernsey, Jersey, Ayrshire and Brown Swiss breeds was studied. These cows were maintained by the Department of Dairy Husbandry and were housed in the University barns. The ration of the cows consisted of alfalfa hay, corn silage and a grain mixture containing 1 per cent trace-mineralized salt. The trace-mineralized salt was com-

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posed of the following minerals: 0.02 per cent cobalt, 0.06 per cent copper, 0.30 per cent iron, 0.007 per cent iodine, 0.15 per cent manganese and 98.75 per cent sodium chloride. The date of parturition for all of the animals was during the months of October and November, 1950.

The vitamin B_{12} content of the milk from the Holstein, Guernsey and Jersey breeds is presented in Table 1. Colostrum samples taken shortly after parturition and one day thereafter were found to be higher in vitamin B_{12} than the milk samples taken during the remainder of the lactation period. Considerable variation in the vitamin B_{12} milk level was observed between animals of the same breed and also between samples from the same cow taken at different stages of lactation.

The vitamin B_{12} content of the colostrum and milk of two Ayrshire and two Brown Swiss cows was found to fall within the range of the breeds presented in Table 1. The vitamin B_{12} level in the milk of one Aberdeen-Angus cow was found to be 3 γ per liter.

The folic acid content (total free *S. faecalis* activity) of milk from the Holstein, Guernsey and Jersey breeds is presented in Table 2. As in the case of vitamin B_{12}, the folic acid level of colostrum also was observed to be high for all three breeds.

The free *Leuconostoc citrovorum* factor activity of some of the milk samples was determined microbiologically using *Leuconostoc citrovorum* as the test organism and crystalline Leucovorin (Lederle) as the reference standard. The acidimetric method was employed using the medium of Sauberlich and Baumann (6), modified by replacing the amino acid mixture with acid-hydrolyzed casein. Since folic acid stimulates the acid production of this organism, sufficient folic acid was added to the medium to make the ratio of sample folic acid to medium folic acid very small. Thus, when 0.05 γ of folic acid was added per milliliter of double-strength medium in both the sample and standard tubes, the small amount of folic acid present in the samples was not believed to have influenced acid production. Because of the presence of only a small amount of *Leuconostoc citrovorum* activity, it was difficult to determine the level of this factor with accuracy; however, its content in the milk of the Holstein, Guernsey and Jersey breeds appeared to be less than 20 per cent of the free folic acid content (total *Streptococcus faecalis* R activity). Sauberlich and Baumann (6) have reported pasteurized cow's milk to contain a very slight amount of this factor.

The vitamin B_{12} content of raw market milk from 23 to 57 individual herds from the vicinity of Madison is presented in Table 3. The samples were obtained at the University Creamery at the time when the mixed milk of each herd was being weighed prior to processing. A wide range of vitamin B_{12} levels was observed within these herds. Also, the milk from the individual herd frequently was found to vary from month to month. The vitamin B_{12} content of pasteurized bottled milk was observed to remain constant from January to August and did not indicate a seasonal variation (Table 3). Since the average vitamin B_{12} level of pasteurized cow's milk is similar to that for raw cow's milk, the pasteurization process does not appear to inactivate this vitamin. This is in agreement with the

TABLE 1

The vitamin B_{12} content of cow's milk[a]

Post-partum	Hostein		Guernsey		Jersey	
	Mean	Range	Mean	Range	Mean	Range
	γ/l.	γ/l.	γ/l.	γ/l.	γ/l.	γ/l.
0 d.	11.2 ± 3.3[b]	7.6-16.4 (7)[c]	19.5 ± 16.0[b]	8.8-38.0 (3)[c]	6.2	5.8- 6.5 (2)[c]
1 d.	8.5 ± 5.8	2.0-23.4 (12)	10.7 ± 5.0	5.5-19.0 (6)	10.6 ± 13.5[b]	1.0-30.0 (4)
2 d.	3.3 ± 2.2	1.2- 7.6 (7)	6.9 ± 3.7	3.4-11.0 (4)	3.5 ± 2.2	1.1- 5.5 (3)
3 d.	3.5 ± 2.1	0.9- 8.2 (11)	6.0 ± 2.6	2.9- 9.1 (5)	4.0 ± 4.1	0.8- 9.4 (4)
1 wk.	4.6 ± 3.3	1.5-10.3 (9)	4.8 ± 0.0	4.8- 4.9 (3)	4.1 ± 2.4	0.7- 6.8 (6)
2 wk.	3.8 ± 2.1	1.1- 8.0 (10)	4.7 ± 1.7	2.6- 7.3 (5)	3.4 ± 1.4	2.2- 6.3 (6)
3 wk.	2.7 ± 1.5	0.7- 5.6 (9)	5.8 ± 1.5	3.6- 6.8 (4)	2.8 ± 1.0	1.4- 4.0 (6)
4 wk.	2.7 ± 1.4	2.0- 5.1 (5)	4.6 ± 1.9	2.7- 7.1 (5)	3.4 ± 1.8	2.2- 5.5 (3)
10 wk.	4.4 ± 2.7	1.8-11.5 (12)	5.2 ± 1.8	2.5- 7.7 (5)	2.8 ± 0.4	2.4- 3.4 (6)
20 wk.	3.7 ± 1.6	1.1- 6.1 (12)	6.1 ± 1.2	5.4- 8.2 (5)	6.5 ± 1.7	4.5- 8.3 (5)

[a]A total of 12 Holstein, 6 Guernsey, and 6 Jersey cows were used in this study.
[b]Standard deviation.
[c]Figures in parentheses represent the number of cows.

TABLE 2

The folic acid content of cow's milk[a]

Post-partum	Hostein		Guernsey		Jersey	
	Mean	Range	Mean	Range	Mean	Range
	γ/l.	γ/l.	γ/l.	γ/l.	γ/l.	γ/l.
0 d.	21.2 ± 16.4[b]	9.0-50.0 (5)[c]	30.0 ± 14.0[b]	14.0-38.0 (3)[c]	.....	.....
1 d.	10.2 ± 7.1	2.0-25.0 (11)	18.0 ± 13.0	1.5-34.0 (6)	30.0	22.0-38.0 (2)[c]
2 d.	1.7 ± 1.1	0.5- 3.2 (5)	9.4 ± 9.0	1.8-21.0 (4)	3.8	(1)
3 d.	1.7 ± 1.1	0.5- 4.1 (7)	2.4 ± 1.9	0.6- 5.6 (5)	.....	.....
1 wk.	1.8 ± 1.2	0.3- 3.7 (8)	1.0 ± 0.4	0.5- 1.6 (6)	0.3	0.1- 0.4 (2)
2 wk.	1.5 ± 1.6	0.3- 4.5 (8)	1.1 ± 0.6	0.5- 1.8 (5)	0.9	0.1- 2.1 (3)
3 wk.	1.8 ± 1.7	0.1- 5.3 (7)	1.5 ± 0.5	1.1- 2.1 (4)	0.4	0.3- 0.4 (2)
4 wk.	1.6 ± 1.6	0.3- 2.7 (6)	1.0 ± 0.6	0.4- 1.7 (4)	0.3	(1)
10 wk.	2.8 ± 1.5	0.3- 6.0 (10)	2.3 ± 1.3	1.3- 4.4 (5)	1.5 ± 1.5[b]	0.2- 4.4 (6)
20 wk.	2.2 ± 1.2	0.7- 3.9 (11)	1.6 ± 1.0	0.6- 2.8 (5)	1.6 ± 0.6	0.9- 2.3 (5)

[a]These results are from the same samples used in the vitamin B_{12} study presented in Table 1.
[b]Standard deviation.
[c]Figures in parentheses represent the number of cows.

TABLE 3

The vitamin B_{12} content of market cow's milk

	Raw milk		No. of herds	Pasteurized milk		
	Mean	Range		Mean	Range	No. of samples
	γ/l.	γ/l.		γ/l.	γ/l.	
Jan.	.....	.....	....	4.4 ± 0.5[a]	4.0-4.8	5
Mar.	2.9 ± 1.0[a]	1.3-5.0	39	3.5 ± 0.2	3.2-3.8	5
Apr.	2.6 ± 1.2	1.3-5.4	23	3.4	3.3-3.5	2
May	3.4 ± 0.7	2.2-4.9	24	.....	.....	....
July	3.8 ± 1.3	1.6-6.5	56	.....	.....	....
Aug.	3.0 ± 1.5	0.8-7.6	57	3.7 ± 0.4	3.1-4.0	5

[a] Standard deviation.

results of Bosch, Bixby and Elvehjem (2), who have found that pasteurization does not alter the vitamin B_{12} content of cow's milk.

DISCUSSION

The wide variation in the milk vitamin B_{12} level found to exist during the lactation period emphasizes that conclusions drawn from an insufficient number of sampling days may be misleading.

An explanation for the large variation in the vitamin B_{12} content of cow's milk may be the influence of dietary cobalt upon rumen synthesis of vitamin B_{12}. Cobaltized and trace-mineralized salt fed to ewes was found by Harper, Richard and Collins (5) to increase significantly the vitamin B_{12} level of ewe's milk. In the case of the goat, Collins, Boldt, Elvehjem and Hart (3) have found that trace-mineral feeding increased the milk vitamin B_{12} level only during the first week of the lactation period. No direct information is available concerning the influence of added dietary cobalt upon the vitamin B_{12} content of cow's milk. Since the average vitamin B_{12} level of the mixed raw milk from the University herd (receiving trace-minerals) was found to be 3.6 γ per liter, feeding trace-minerals to this herd did not appear to increase the milk vitamin B_{12} level above that of other herds or of market milk (Table 3).

Anthony *et al.* (1) have reported the level of vitamin B_{12} in the milk of the Holstein and Jersey. Compared to the findings at Wisconsin, these workers observed a higher level of this vitamin in both the colostrum and the milk. They also found a higher level of vitamin B_{12} in Holstein colostrum than in that of the Jersey.

The vitamin B_{12} content of the milk of Indian cows which was reported by Sreenivasamurthy *et al.* (7) was lower than that observed in this study. No breed differences were observed among the three breeds of Indian cows. In agreement with results from this country, the colostrum level of vitamin B_{12} also was found to be higher than that of the regular milk.

SUMMARY

The folic acid and vitamin B_{12} content of cow's colostrum was observed to be higher than that of the regular milk. No differences were observed among the breeds of cows studied.

Measurement of *L. citrovorum* activity in cow's milk showed it to be present in very low concentrations.

The average vitamin B_{12} content of the market milk from 23 to 57 herds was found to range from 2.6 to 3.8 γ per liter. The average vitamin B_{12} level of pasteurized milk was found to vary from 3.4 to 4.4 γ per liter. Neither the raw nor pasteurized milk samples studied appeared to indicate a variation in vitamin B_{12} content due to seasonal differences.

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THE INFLUENCE OF TRACE-MINERALIZED SALT UPON THE VITAMIN B_{12} AND FOLIC ACID CONTENT OF GOAT'S MILK[1]

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The vitamin B_{12} and folic acid content of the milk of goats receiving iodized salt throughout their lactation period was determined by Collins, Harper, Schreiber and Elvehjem in 1951 (1). The average colostrum level of vitamin B_{12} was observed to be 4.2 γ per liter immediately following parturition and was observed to drop rapidly to the low level of 0.18 γ per liter after 3 weeks. Since Harper, Richard and Collins (3) reported that the vitamin B_{12} level of ewe's milk could be increased by the addition of cobalt or trace-minerals (containing cobalt) to the ewe's diet, similar studies were initiated with the dairy goat. The effect of trace-minerals upon the folic acid content of goat's milk also was studied.

EXPERIMENTAL AND RESULTS

Goats of the Saanen breed were used in this study. These animals were housed in the University dairy barn and fed a ration consisting of a grain mixture, corn silage and alfalfa hay. No attempt was made to feed a ration low in cobalt and no cobalt-deficiency symptoms were observed in the goats or the kids. The grain mixture was composed of the following: ground corn, 35.0 per cent; ground oats, 40.0 per cent; wheat bran, 12.5 per cent; soybean oil meal, 10.0 per cent; ground limestone, 0.75 per cent; bone meal, 0.75 per cent; and salt (iodized or trace-mineralized[3]), 1.0 per cent.

The milk samples were frozen immediately after collection. The vitamin B_{12} and folic acid content was determined microbiologically in a manner similar to that described by Collins *et al.* (1).

During the summer of 1950, a daily oral supplement of 50 mg. of cobalt ($CoCl_2 \cdot 6H_2O$) to two lactating goats for a period of 3 weeks failed to alter the milk vitamin B_{12} level. Also, during September of 1950, seven lactating goats were fed 1 per cent trace-mineralized salt (containing 0.02 per cent cobalt) in their grain mixture for a period of 4 weeks. Again, no increase was observed in the vitamin B_{12} content of the goat's milk.

To determine whether trace-mineralized salt would affect the vitamin B_{12} level of the colostrum of goats, eight animals were fed trace-mineralized salt during January of 1951, one month prior to parturition. As Table 1 shows, the goats receiving trace-minerals had a higher level of vitamin B_{12} in their colostrum and milk during the first week of lactation than did the goats receiv-

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[3] See footnote *a* of Table 1 for composition.

TABLE 1

The influence of trace-mineralized salt upon the vitamin B_{12} content of goat's milk

	Days post-partum	Iodized salt		Trace-mineralized salt[a]	
		Mean	Range	Mean	Range
		(γ/l.)	(γ/l.)	(γ/l.)	(γ/l.)
1951	0	4.3 ± 3.5[b]	1.0 –10.0 (5)[c]	14.6 ± 19.3[b]	1.0–63.0 (8)[c]
	1	1.2 ± 0.8	0.2 – 2.4 (5)	5.0 ± 1.8	2.6– 7.9 (8)
	2	0.7 ± 0.6	0.3 – 1.8 (5)	1.7 ± 1.2	0.5– 4.0 (8)
	7	0.3 ± 0.1	0.2 – 0.4 (5)	0.8 ± 0.5	0.2– 1.4 (7)
	21	0.25 ± 0.01	0.1 – 0.5 (5)	0.44 ± 0.4	0.1– 1.4 (7)
	42	0.2 ± 0.02	0.1 – 0.3 (5)	0.2 ± 0.1	0.1– 0.4 (7)
1952	0	2.6 ± 2.0	0.5 – 6.5 (9)	9.3 ± 5.9	3.3–19.0 (6)
	1	0.5 ± 0.3	0.2 – 1.2 (8)	1.3 ± 0.7	0.6– 2.5 (6)
	2	0.2 ± 0.1	0.1 – 0.4 (8)	0.8 ± 0.6	0.3– 1.7 (5)
	7	0.16 ± 0.1	0.1 – 0.4 (8)	0.6 ± 0.7	0.2– 1.9 (5)
	21	0.1 ± 0.03	0.07– 0.15 (7)	0.3 ± 0.2	0.1– 0.6 (5)
	42	0.1 ± 0.04	0.05– 0.16 (5)	0.35 ± 0.06	0.3– 0.4 (3)

[a] The trace-mineralized salt was composed of the following minerals: 0.02% Co, 0.06% Cu, 0.30% Fe, 0.007% I, 0.15% Mn and 98.75% NaCl.
[b] Standard deviation.
[c] Figures in parentheses represent the number of animals.

ing iodized salt; however, after this time no differences were observed. In January, 1952, the influence of trace-mineralized salt upon the vitamin B_{12} level of colostrum again was studied. The data shown in Table 1 confirm the previous year's results.

Since the vitamin B_{12} content of goat's milk varies so greatly among animals receiving the same treatment, the effect of trace-mineralized salt is seen more clearly when each goat acts as its own control. In Table 2 all goats which

TABLE 2

The influence of trace-mineralized salt upon the vitamin B_{12} content of milk of individual goats (γ of vitamin B_{12}/l.).

Treatment	Post-partum	Goat No.							
		1	2	3	4	5	6	7	Mean
	(d.)								
Iodized salt 1950	0	.....	....	....	2.0	.....	1.6	.....	1.8
	1	.....	....	0.9	0.5	1.2	0.6	.....	0.8
	2	.....	....	0.8	0.4	0.5	0.4	.....	0.5
	7	.....	....	0.5	0.3	0.4	0.3	.....	0.4
	21	.....	....	0.2	0.3	0.2	0.1	.....	0.2
	42	.....	....	0.1	0.1	0.1	0.2	.....	0.1
Trace-mineralized salt 1951	0	15.0	1.0	6.5	8.5	11.0	4.0	64.0	15.7
	1	5.5	2.6	3.4	4.6	5.0	4.6	6.5	4.6
	2	1.6	0.6	1.0	0.5	1.4	4.0	3.0	1.7
	7	1.4	0.3	0.7	0.2	1.4	0.5	1.2	0.8
	21	0.5	0.3	0.5	0.2	0.1	0.2	1.4	0.5
	42	0.3	....	0.1	....	0.1	0.1	0.4	0.2
Iodized salt 1952	0	3.2	2.5	4.3	0.5	.....	3.3	6.5	3.4
	1	0.4	0.3	1.2	0.4	.....	0.3	0.7	0.5
	2	0.2	0.2	0.3	0.2	.....	0.1	0.4	0.3
	7	0.2	0.1	0.2	0.1	.....	0.1	0.4	0.2
	21	0.1	0.1	0.1	0.1	.....	0.1	0.1	0.1
	42	.....	0.1	....	....	.....	0.1	0.2	0.1

were subjected to both the iodized salt and the trace-mineralized salt treatments are presented. It appears rather definite that trace-mineralized salt (containing cobalt) does increase the vitamin B_{12} content of goat's milk during the first week of lactation and in some cases for as long as 3 weeks.

The free folic acid content of the goat's milk was not found to be influenced by the trace-mineralized salt diet during the 1951 and 1952 experiments. The results for 1951 are presented in Table 3. In a similar manner Harper *et al.* (4) observed that the folic acid content of ewe's milk was not affected by the addition of cobalt or trace-minerals to the ewe's diet.

TABLE 3

The folic acid content of goat's milk during 1951

Post-partum	Iodized salt		Trace-mineralized salt	
	Mean	Range	Mean	Range
(d.)	*(γ/l.)*	*(γ/l.)*	*(γ/l.)*	*(γ/l.)*
0	4.3	1.9–7.8 (5)[a]	6.0	1.3–15.0 (6)[a]
1	1.0	0.1–3.5 (5)	2.3	0.7– 5.0 (4)
2	0.8	0.0–1.3 (5)	0.8	0.3– 1.5 (6)
7	0.3	0.0–1.4 (5)	0.5	0.1– 1.4 (6)
21	0.4	0.0–1.6 (5)	0.15	0.0– 0.5 (4)

[a] Figures in parentheses represent the number of animals.

DISCUSSION

The increase in the vitamin B_{12} content of goat's colostrum, which resulted from feeding trace-mineralized salt, is believed to be due to increased rumen synthesis of vitamin B_{12} produced by the cobalt component of the trace-minerals.

Although Couch *et al.* (2) have observed the vitamin B_{12} level of goat's and cow's blood to be comparable, Collins *et al.* (1) have found the vitamin B_{12} level of goat's milk to be very low in comparison to that of cow's milk. Thus, it would appear that the milk-forming cells of the mammary gland of the goat do not have the ability to concentrate the blood vitamin B_{12}. The close correlation between vitamin B_{12} and nitrogen content of goat's colostrum and milk (1) suggests that the milk proteins may control the level of vitamin B_{12} in goat's milk.

The vitamin B_{12} content of the goat's colostrum collected on the day of parturition was observed to be very high. Samples taken on the first day post-partum exhibited much lower values. Thus, it seems probable that the extreme variation in the *0* day values is due to a difference in the number of hours after parturition at which sampling was done.

The colostrum and milk level of folic acid was observed to be lower during the 1951 and 1952 studies than that reported initially by Collins *et al.* (1). The capacity of the microorganisms present in milk to synthesize folic acid activity was recognized and was believed to have influenced the results of this first study (1). The more recent lower level of folic acid is believed to be more accurate, since the milk samples were maintained in a frozen state until analyzed in order to prevent microbial activity. In spite of a lower level of folic acid,

the average folic acid content of colostrum still appears to be greater than that of the milk (Table 3).

SUMMARY

Goats receiving trace-mineralized salt (containing cobalt), in comparison to those receiving only iodized salt, were observed to have a higher level of vitamin B_{12} in their colostrum and milk during the first week post-partum. Trace-mineralized salt or a 50-mg. supplement of cobalt per goat per day had no influence upon the level of this vitamin in the goat's milk after this time.

The addition of trace-minerals to the diet of the goat did not influence the free folic acid level of the goat's milk. The lower folic acid content of goat's milk observed in this study is believed to be more accurate than the previous data of Collins *et al.* (1).

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PROTEIN-BOUND IODINE LEVELS IN DAIRY CATTLE PLASMA[1,2]

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Today's economic situation demands that we obtain the greatest possible efficiency in the production of milk, butterfat and meat from our dairy animals. This cannot be done unless we thoroughly understand the physiological functioning of cattle and so select and manage them as to utilize fully their productive potentials.

A long step forward would be taken if some means could be developed whereby the future worth of an animal could be estimated while it was still a calf. An endocrine analysis may offer some possibilities in this direction. Unfortunately no suitable assay method exists for some of the hormones, but the rate of secretion of the thyroid hormone, which has been shown to be related to dairy cattle performance, is closely related to the plasma level of protein-bound iodine in many species. It has been a useful tool in estimating thyroid function in human medicine and is closely related to thyroid activity in rats, mice, dogs, rabbits and other laboratory animals. Little information is available, however, on the plasma protein-bound iodine (PBI) concentration of dairy cattle and its relation to the functional condition of their thyroid glands.

As an approach to this problem, the plasma PBI levels of dairy animals of various ages and breeds have been determined in a relatively large number of cases. It was hoped that such data would provide a basis for establishing a normal range of PBI values. If such a range could be established, the influence of various environmental factors and the relation of plasma PBI concentration to performance could be determined in future studies.

HISTORICAL

Although values have been reported for the plasma PBI concentration of dairy cattle (3, 8), there have been only two studies involving a significant number of animals.

Long *et al.* (6) recently reported an average of 3.15 γ of iodine per 100 ml. of plasma in dairy and beef animals. The values arranged according to breed were: Jersey, 4.11; Guernsey, 3.51; Brown Swiss, 3.37; Ayrshire, 3.19; Holstein, 2.73 and beef breeds, 2.19 γ per cent. Some of the breed differences were statistically significant. Calves averaged 4.8 γ per cent, 3- to 4-year old cows averaged 3.1 and 7- to 8-year old animals averaged 2.6 γ per cent. Thus, there appeared to be a decrease in PBI concentration with increasing age.

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Reece and Man (7) found an average plasma PBI level of 4.6 and 3.7 γ per cent for non-pregnant, lactating Jersey and Brown Swiss cows between 3 and 7 years of age. Calves of the two breeds ranged from 4.2 to 11.9 and 4.5 to 8.7 γ per cent, respectively. Four beef animals averaged 4.2, while paired Jerseys averaged 5.0 γ per cent.

On the basis of these limited data, it appears that breed and age differences in plasma PBI concentration may exist in dairy cattle.

MATERIALS AND METHODS

Plasma samples used in this study were obtained from Holstein, Jersey, Guernsey, Ayrshire and Brown Swiss cows and calves of the Michigan State College dairy herds, none of which received supplemental iodine in their rations. The blood was drawn from the jugular vein directly into a 15-ml. centrifuge tube containing 4 drops of 20 per cent sodium oxalate as an anti-coagulant. The blood was centrifuged as soon as possible after withdrawal and placed in a refrigerator until the iodine determination could be made.

The PBI determinations were made according to the distillation method described by Barker (1) and Barker and Lipner (2), with slight modifications (5). This technique involves the separation of the PBI fraction from the inorganic iodine by precipitation and washing. The precipitate then is digested and oxidized with chromic oxide and H_2SO_4 leaving the iodine in a highly oxidized state. The exact form of the iodine is not known but it is thought to be iodic acid.

The iodic acid then is reduced with phosphorous acid and the volatile iodine (hydrogen iodide or elemental iodine) is distilled off and collected in a sodium hydroxide-arsenious acid solution. The colorimetric determination of iodine is based on its catalytic effect on decolorization of ceric sulfate by arsenious acid.

RESULTS AND DISCUSSION

Age differences. Between June 12, 1951, and March 3, 1952, 296 determinations of the plasma PBI concentrations were made on 177 cows and calves in the Michigan State College dairy herds. The results, arranged according to age of the animals, are shown in Table 1.

The 296 determinations averaged 6.8 γ per cent. An inspection of these data reveals, however, that an average value, not qualified by a consideration of age, had little meaning, since the plasma PBI concentration tended to decrease with

TABLE 1

Plasma PBI concentrations in cattle of different ages

Age	No. of animals	No. of determinations	Range	Av.
			(γ %)	(γ %)
Under 48 hr.	30	30	6.0 - 29.7	13.7 ± 5.0
3 d. to 12 mo.	91	114	2.7 - 18.0	7.2 ± 3.2
13 mo. to 24 mo.	26	60	3.0 - 15.3	6.2 ± 2.3
25 mo. or over	30	92	2.1 - 16.5	4.6 ± 1.6

age. Thus, calves averaged 13.7 γ per cent of plasma PBI during the first 2 days after birth. During the rest of the first 12 months the average concentration was 7.2 γ per cent. From 13 to 24 months the average was 6.2 γ per cent, while the level in cows over 24 months was 4.6 γ per cent.

Although these averages showed distinct age differences, there was a considerable amount of overlapping in the range of values between the different age groups. The values of calves under 48 hours of age only once fell below 8.0 γ per cent and seldom rose above 18.0 γ per cent. Within these limits the distribution was fairly regular. An abrupt decrease in the average plasma PBI concentration occurred soon after the second day. The values of calves between 48 hours and 12 months of age fell as low as 2.7 γ per cent, and 84 per cent had concentrations less than 10.0 γ per cent. The downward trend of PBI concentration continued in heifers between 12 and 24 months of age with 85.0 per cent of the values below 8.0 γ per cent. The plasma PBI concentrations of cows over 24 months of age were generally still lower. In this group nearly 90 per cent of the values were less than 6.0 γ per cent.

In view of these data it is suggested that certain ranges of plasma PBI concentrations be considered normal for animals of each of the different age groups. These suggested ranges are shown in Table 2 and were so selected as to include 85 to 90 per cent of the determinations in each case.

TABLE 2

Normal range of plasma PBI concentrations of dairy animals at different ages

Age	No. of determinations	Observed range	Suggested normal range	% of determinations included
		(γ %)	(γ %)	
Under 48 hr.	30	6.0 - 29.7	8.0 - 18.0	86.7
3 d. to 12 mo.	114	2.7 - 18.0	3.5 - 12.0	87.7
13 mo. to 24 mo.	60	3.0 - 15.3	3.5 - 10.0	85.0
25 mo. or over	92	2.1 - 16.5	3.0 - 8.0	87.0

Since no studies of the effect of hypo- or hyperthyroidism on the plasma PBI level in cattle have been conducted, it is impossible to relate these pathological conditions to the suggested ranges. It may be found that these ranges can be considerably enlarged before thyroid dysfunction is encountered. It is known that cows receiving thyroprotein (protamone) at the rate of one gram daily per 100 lb. of body weight (equivalent to hyperthyroidism) show PBI concentrations of over 20.0 γ per cent (4). Undoubtedly, as the relationship of plasma PBI to thyroid activity is better understood, some revision of these ranges will become advisable.

Breed differences. Plasma samples were obtained on four occasions from seven Jersey and nine Holstein females from one of the College herds. The collection dates (June, August, November and March) correspond roughly with the four seasons, although they were not selected with this in mind. All of the

animals were over 18 months of age and, with the exception of K131 and K132, were in milk during this period.

The results of the PBI determinations are shown in Table 3. The Jerseys averaged 4.97 γ per cent, while the Holsteins averaged 4.84 γ per cent.

Statistical analysis showed that no significant differences existed between the breeds or cows within the breeds. Although the values for both breeds tended to be lowest at the November sampling, the seasonal differences were statistically significant only for the Holsteins. Undoubtedly, any seasonal effects were minimized by the fact that these animals received only dry-lot exercise instead of pasture and were fed only hay and concentrate mix.

TABLE 3

Plasma PBI levels in Jersey and Holstein females over 18 mo. old

Herd no.	Breed	Age	Sampling dates				Av.
			6-12-51	8-14-51	11-12-51	3-18-52	
		(mo.)	*(γ %)*	*(γ %)*	*(γ %)*	*(γ %)*	*(γ %)*
K 5	J	72	5.1	4.8	3.8	4.1	4.45 ± 0.28
K 10	J	72	4.8	4.2	4.9	4.4	4.58 ± 0.13
K 101	J	54	6.9	6.0	4.5	4.8	5.55 ± 0.88
K 104	J	52	3.6	5.7	3.6	5.4	4.65 ± 0.27
K 105	J	47	5.1	5.1	5.0	4.9	5.02 ± 0.06
K 115	J	34	4.8	4.5	4.1	6.7	5.02 ± 0.05
K 132	J	22	6.0	6.6	4.4	5.4	5.60 ± 0.66
	Average		5.19 ± 0.87	5.27 ± 0.66	4.33 ± 0.23	5.10 ± 0.62	4.97 ± 0.76
K 15	H	72	5.7	4.8	3.3	4.1	4.50 ± 0.56
K 17	H	66	3.0	5.4	3.8	3.7	3.98 ± 0.73
K 18	H	68	5.1	8.1	3.8	4.7	5.42 ± 2.66
K 19	H	48	3.0	3.0	4.4	4.6	3.75 ± 0.57
K 20	H	55	3.9	5.1	3.0	6.0	4.50 ± 0.31
K 109	H	44	4.2	5.7	4.2	5.0	4.78 ± 0.34
K 128	H	23	5.7	5.4	4.1	3.1	4.58 ± 1.04
K 131	H	19	7.5	7.2	5.2	4.8	6.18 ± 1.35
K 134	H	19	8.4	6.3	5.1	3.9	5.92 ± 2.82
	Average		5.17 ± 3.12	5.67 ± 1.86	4.10 ± 0.48	4.44 ± 0.58	4.84 ± 1.93

The considerable variation in iodine concentration of the same cow on different dates leads one to wonder if the variability was due to a real difference or if it was merely a reflection of the inadequacy of a single sample for expressing a cow's PBI level. In order to check this point, samples were drawn from a cow on six consecutive days. The PBI values obtained averaged 3.65 ± 0.32 γ per cent, which is no greater than the variability of multiple determinations on the same sample. Therefore, a single determination is sufficient to show the plasma PBI concentration.

The possibility of herd differences in the plasma PBI level was indicated when the concentration of a group of inbred Jersey females purchased in California and transported to Michigan was compared with that of the Jersey females in the College herd. Whereas the cows raised in the College herd averaged 4.97 ± 0.76 γ per cent, the level in 14 females from California averaged

3.3 ± 0.38 γ per cent on the basis of two determinations made one and three months after their arrival. This difference was statistically significant.

Whether the difference between the two groups was due to genetic or environmental factors is not known. It might be postulated that cows from areas bordering the sea coast (where the iodine content of the feed probably is adequate to meet the animal's needs) have thyroid glands which are not immediately capable of secreting normal amounts of hormone when the cow is moved to an iodine-deficient area and placed on a ration containing no supplemental iodine (iodized salt) as were these cows. This question may be resolved when later PBI determinations are made. If the California cows continue to be lower than those in the regular herd, the variation probably is due to a real genetic difference.

Relation to sterility. Plasma PBI determinations were made on a group of 19 cows under study by the Department of Pathology as difficult breeders. Although all cows had histories of many breedings without conceptions, four had been declared pregnant by a veterinarian at the time of sampling. In some cases only one sample was taken, but as many as five were taken in others.

The average plasma PBI concentration of 3.7 ± 0.93 γ per cent was significantly lower than that of the Jersey and Holstein cows in the College herd but was similar to that of the California Jerseys.

It is not yet clear whether or not the lower values of the problem cows were related to their low fertility. Three possibilities suggest themselves: In some cases at least, it may be that low breeding efficiency is the result of a hypogonadic condition which also causes a lowered plasma PBI level. On the other hand, thyroid hypofunction and consequent low plasma PBI may induce reproductive inefficiency. Thirdly, in spite of the known thyroid-ovarian interactions no specific relationship between thyroid activity and fertility of cows has been shown; therefore, the low plasma PBI values found may have been entirely unrelated to the sterility of these animals.

Since the completion of the work described in this report, Reece and Man (7) have presented data which they believe indicate that the plasma PBI concentration may not reflect accurately the circulatory level of the thyroid hormone when iodized salt is fed to dairy cattle. They suggest that differences between animals of less than 1.6 γ per cent may be due to the iodine content of the ingested salt.

No reports on the effect of the use of iodized salt on the plasma PBI level of cattle have come to the attention of the writer, although it is known that the amounts of iodine used to treat hypothyroidism and Graves disease in humans cause a marked rise in the plasma PBI concentration. In herds where iodized salt is supplied, it is possible that part of the organic iodine in the plasma may be of non-thyroidal origin. On the other hand, where dairy animals are not given supplemental iodine (as was the case in the present work), there is no apparent reason why the nature of the plasma PBI fraction should be different from other species.

It would seem, too, that the similarities in the results obtained in the present work to the findings of Long *et al.* (6) and Reece and Man (7) preclude an effect of normal levels of iodine supplementation sufficient to prevent the use of PBI determinations in herds receiving iodized salt. It is hoped that iodine feeding experiments now underway in this laboratory will resolve this question.

SUMMARY AND CONCLUSIONS

A method for estimating thyroid activity through determination of the plasma concentration of protein-bound iodine has been applied to dairy cattle. A considerable amount of variation was found between animals and within animals at different times. The PBI concentration tended to decrease with age. Calves under 48 hours of age averaged 13.7 γ per cent of plasma PBI, while those between 2 days and 12 months averaged 7.2 γ per cent. Heifers between 1 and 2 years of age averaged 6.2 and older cows 4.6 γ per cent, respectively.

It is suggested that normal ranges of PBI concentrations of dairy animals of various ages be tentatively set as follows: Calves under 2 days, 8.0 to 18.0 γ per cent; animals between 2 days and 12 months, 3.5 to 12.0 γ per cent; animals 13 to 24 months of age, 3.5 to 10.0 γ per cent; and cows over 24 months 3.0 to 8.0 γ per cent.

A group of Jersey cows maintained in the Michigan State College dairy herd averaged 4.97 γ per cent, while Holsteins in the same herd averaged 4.84 γ per cent of plasma PBI. This difference was not significant. Cows of both breeds had lower PBI concentrations in November, than in June, August, or March. The seasonal variations were statistically significant only for the Holstein cows, however.

A herd of Jersey cows brought from California and a group of cows under study as problem breeders had similar PBI concentrations of 3.3 and 3.7 γ per cent, which were significantly lower than those of the cows in the regular College herd. Possible reasons for these low concentrations are discussed.

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BLOOD LEVELS OF SOME INORGANIC AND ORGANIC CONSTITUENTS IN NORMAL PARTURIENT COWS AND COWS WITH PARTURIENT PARESIS[1,2,3]

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The literature on parturient paresis contains many reports concerning the levels of blood constituents occurring prior to or during the attacks of this disease. It is well established that a decrease of calcium and phosphorus and an increase of magnesium in the blood are characteristic of milk fever. Studies of the organic constituents in the blood associated with the disease have been few and limited to a small number of cows. The literature on milk fever has recently been reviewed in great detail by Hibbs (5) in this journal; therefore, it is unnecessary to review the literature in this paper.

EXPERIMENTAL METHODS

All the cows in this experiment were from the dairy herd of The State College of Washington. All the cows described as having milk fever were diagnosed by a veterinarian as having typical parturient paresis. A blood sample was drawn from the external jugular vein immediately before treatment. Heparin was used as the anticoagulant in those samples on which whole blood analyses were made. Further blood samples were taken on about the fifth, tenth, fifteenth and thirtieth days following treatment. By way of control, the same procedure was followed with three cows which had normal parturitions during the same period.

Methods used for calcium, sodium, potassium and chloride determinations were the same as those reported previously (10). Pyruvic acid was determined according to the method of Friedman (4), lactic acid by the method of Barker and Summerson (1), citric acid by the method of Taussky and Shorr (7) and hematocrits according to the method of Wintrobe (11). Analyses for the organic constituents studied were started within 2 to 3 hours after drawing the samples.

RESULTS

The data obtained from blood analyses for cows which developed milk fever are presented in Table 1. Data for normally calving cows are shown in Table 2.

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TABLE 1

Blood levels of calcium, sodium, potassium, chlorides, pyruvic acid, lactic acid, citric acid and hematocrit in cows with parturient paresis

Cow No.	Date	Blood serum calcium	Whole blood sodium	Whole blood potassium	Blood serum chlorides	Whole blood pyruvic acid	Whole blood lactic acid	Blood serum citric acid	Hematocrit
		(mg%)	*(mg%)*	*(mg%)*	*(mg%)*	*(mg%)*	*(mg%)*	*(mg%)*	*(%)*
244[a]	1/4	6.1	256	61.3	333	2.30	.....	5.3	44.8
	1/8	6.5	246	58.0	310	1.30	82.2	2.9	39.1
	1/14	8.6	249	52.6	323	1.53	27.7	4.3	.....
	1/21	10.3	.....	56.2	318	1.16	22.2	5.0	40.5
	2/4	10.4	270	51.8	404	1.30	13.2	4.2	39.8
266	1/9	4.4	240	53.3	.....	2.86	140.4	2.0	45.0
	1/17	9.7	256	48.5	324	0.64	27.9	3.7	39.4
	1/21	8.8	232	48.2	318	0.83	18.4	5.1	40.0
	1/25	9.6	.....	.....	317	.....	.....	3.3	38.0
	2/12	9.7	277	45.3	361	1.06	12.1	6.8	34.8
243	2/12	4.2	246	47.4	346	1.90	82.8	2.8	48.5
	2/14	7.9	246	47.3	362	1.15	75.1	2.9	.....
	2/16	8.4	262	44.0	337	0.97	25.9	3.9	43.2
	2/22	9.5	294	43.6	380	0.87	14.7	3.7	.....
	3/6	10.6	261	44.0	362	0.78	35.9	5.7	39.8
	3/8	8.2	252	39.9	300	0.93	.....	1.8	38.5
237	2/28[b]	4.8	251	55.6	374	.....	.....	5.6	49.7
	3/6	11.3	253	41.1	382	0.92	58.0	4.8	38.7
	3/14	10.0	302	45.4	358	1.16	52.5	4.7	37.7
	3/22	10.0	252	44.0	338	.....	129.0	5.4	.....
278	3/9[b]	5.6	246	48.5	389	1.88	17.6	7.0	47.5
	3/10[c]	5.6	244	62.7	389	1.65	48.0	2.8	45.0
	3/14	10.0	255	44.4	358	1.50	170.0	4.8	40.0
	3/22	11.2	258	40.5	327	.....	28.9	....	.....
	4/8	.....	261	57.7	357	1.06	32.8	....	41.2
259	4/1	6.8	253	40.8	379	2.80	175.4	1.5	42.0
	4/8	10.9	.....	50.8	336	1.60	40.1	4.9	44.0
	4/13	11.4	254	62.7	355	1.01	18.8	5.9	39.7
	5/2	11.5	.....	.....	.....	1.60	.....	2.8	.....
245	4/8	4.5	246	51.9	396	1.63	90.5	1.8	39.5
	4/9[c]	4.1	164	48.5	368	1.44	46.0	1.7	.....
	4/13	10.6	260	48.5	379	1.01	24.3	3.2	35.0
	4/18	10.5	266	42.7	.....	.....	12.2	....	.....
	5/2	11.6	.....	67.6	.....	1.70	.....	4.7	.....
2116	1/28								
	5 pm	6.1	270	52.4	.....	1.52	27.4	1.7	39.5
	10 pm[c]	3.3	266	53.1	372	3.30	42.1	2.0	45.0
	2/1	10.5	262	40.1	395	1.38	.....	....	36.2
	2/7	10.8	264	47.1	393	0.90	9.9	4.6	37.2
	2/12	10.7	263	43.9	367	0.99	15.9	4.9	36.0
	2/27	10.3	274	49.7	364	1.01	9.9	6.3	39.5
Av. pre-treatment level		5.0	244	52.3	372	2.13	74.5	3.1	44.6
4-6 d. post-treatment		9.7	256	46.0	360	1.24	73.6	4.1	38.8
8-14 d. post-treatment		10.0	265	47.8	347	1.02	24.1	4.5	38.8
15-22 d. post-treatment		10.4	259	47.0	340	0.98	25.7	4.7	36.1
24-32 d. post-treatment		10.2	267	52.0	357	1.24	39.4	4.6	38.8

[a] 1st date for each cow indicates pretreatment levels.
[b] Developed parturient paresis prepartum.
[c] Cow relapsed and was treated a 2nd time.

TABLE 2

Blood levels of calcium, sodium, potassium, chlorides, pyruvic acid, lactic acid, citric acid and hematocrit in normally calving cows

Cow No.	Date	Blood serum calcium	Whole blood sodium	Whole blood potassium	Blood serum chlorides	Whole blood pyruvic acid	Whole blood lactic acid	Blood serum citric acid	Hematocrit
		(mg%)	*(mg%)*	*(mg%)*	*(mg%)*	*(mg%)*	*(mg%)*	*(mg%)*	*(%)*
2117	12/29	7.9	246	69.1	434	1.38	14.4	3.7	44.1
	1/2	10.4	265	67.6	421	0.70	35.1	3.9	40.5
	1/7	10.7	241	62.9	323	0.88	35.9	3.4	41.3
	1/13	8.0	256	63.1	325	1.07	30.7	6.0	44.8
	1/28	9.4	260	50.8	359	0.80	19.1	3.6	.....
2118	1/5	8.3	244	64.1	341	1.47	30.6	1.9	42.9
	1/10	9.8	230	59.0	295	1.08	27.0	3.6	46.8
	1/16	11.1	262	60.4	343	1.28	35.4	4.7	40.0
	1/20	8.3	279	59.8	334	1.01	27.2	5.8	46.8
	2/4	9.0	259	52.2	362	1.30	.....	4.3	42.0
299	3/12	7.0	249	51.0	372	1.15	20.4	5.2	39.2
	3/16	10.9	266	44.3	346	1.40	14.7	3.2	44.0
	3/21	10.0	270	44.5	322	1.10	18.7	5.1	41.8
	3/26[a]	10.8	264	51.2	306	1.62	19.8	3.9	42.8
	4/8	10.4	266	41.9	366	1.05	17.5	7.0	36.5
Av. day of calving		7.7	246	61.4	382	1.33	21.8	3.6	42.1
5 d. post-partum		10.4	254	57.0	354	1.06	25.6	3.6	43.8
10 d. post-partum		10.6	259	55.9	329	1.09	30.0	4.0	41.0
15 d. post-partum		9.0	266	58.0	322	1.23	25.9	5.2	44.8
30 d. post-partum		9.6	262	48.3	362	1.05	18.3	5.0	39.2

[a] Developed ketosis.

At the end of each table are presented the average blood levels for the groups at the five periods at which samples were taken.

The blood serum calcium levels are similar to the results described for many previous studies. All values were low for pretreatment blood samples. Cows 2116, 278 and 245 had relapses and were treated a second time. Cows 278 and 237 had parturient paresis both pre and postpartum. The blood serum calcium levels were as low or lower for these cows at the time of the second treatment as at the time of the first treatment.

The whole blood sodium levels for five of the eight cows were somewhat lower in the pre-treatment samples than in subsequent samples. The average value, 244 mg. per cent, for all pre-treatment samples, is lower than the average for any of the post-treatment samples.

The whole blood potassium values for five of the cows are higher in the pre-treatment samples than in subsequent samples, and the average value is higher for pre-treatment samples than the average for any samples drawn subsequent to treatment.

The results for blood serum chloride analyses are similar to those for potassium, *i.e.*, the average pre-treatment level is higher than at any subsequent period, although all cows do not respond in this way.

Further examination of Table 1 reveals marked differences in the blood levels of organic constituents prior to treatment as compared with the concen-

trations found following recovery. Pyruvic acid levels were without exception higher, lactic acid levels were in most cases much higher, citric acid levels were with three exceptions decreased, and hematocrits were higher in pre-treatment samples than in subsequent samples. The average values accentuate this trend.

Table 2 presents the data for three cows which had uncomplicated parturitions. The blood serum calcium levels for this group were lower at parturition than at other times, but not as low as the levels found in the cows suffering from milk fever. Whole blood sodium levels were lower, and potassium and chloride concentration were higher on the day of calving than at subsequent times. The pyruvic acid concentration was slightly higher for this group at this time than at other periods. The levels of lactic and citric acid and hematocrits were essentially the same regardless of the time at which samples were taken.

DISCUSSION

The blood serum calcium values corresponded to those already well established for the conditions described. The low sodium and high potassium and chloride values obtained for the two groups of cows either prior to an attack of milk fever or subsequent to freshening in the non-affected group indicate that this response is not the result of parturient paresis. The changes in sodium, potassium and chloride values are not so consistent nor are they so drastic as the changes in blood calcium. It is possible that the changes observed in the averages of the first three elements may be a coincidence, or further study may reveal that real differences in the concentration of these elements are associated with this disease. However, changes of 40 to 50 per cent, such as observed in blood calcium, obviously do not occur in the concentrations of sodium, potassium and chlorides coincident with milk fever attacks. The latter three elements have been shown to respond in a similar way during normal or uncomplicated parturitions (9). Thus, it may be that as calcium decreases, so does sodium, and that small increases in potassium and chlorides also occur at the time of parturition. A physiological explanation for this change is not apparent from the data available.

The lower blood sodium and higher blood potassium levels might suggest an adrenal involvement of all cows at parturition. Urinary studies on similar cases, however, lend little support to such an hypothesis (9).

There does appear to be a definite difference between the blood levels of pyruvic, lactic and citric acids for those cows which developed milk fever and those cows which calved normally. Blosser and Smith (2) found that serum citric acid levels decreased in all cows at the time of parturition with a more precipitous drop in cows affected with milk fever as compared with normal cows. The high levels of pyruvic acid prior to treatment represented the largest and most consistent changes in blood levels associated with parturient paresis, aside from the changes in calcium levels. It is worthy of note that in two of three milk-fever cows having high serum citric acid in the pre-treatment period, the attack of milk fever occurred prepartum. Insufficient evidence is available to indicate whether or not this is the result to be expected. None of the other con-

stituents studied appeared to be different in these two prepartum cases. The observation that blood levels of lactic acid are higher in cows with parturient paresis confirms the studies of vandenBurg (8).

The significance of an increased pyruvic and lactic acid and decreased citric acid, if such is a true characteristic of parturient paresis, is difficult to explain in the light of the present theories. One possibility would be that the glycolytic cycle of metabolism is interrupted, since pyruvic acid and its equilibrium product, lactic acid, presumably combine with the so-called 2-carbon fragment to form citric acid. An enzyme catalyzing this condensation has been isolated recently by Stern and Ochoa (6). These studies need to be carried out on a larger number of normal animals in order to have valid comparisons.

The generally higher hematocrit values may indicate a hemoconcentration associated with milk fever. Fish (3) reported a 14 per cent increase in red blood cells in milk-fever cows. This change should result in higher percentages for serum constituents and lower percentages of whole blood concentrations if other factors were not involved. The changes noted in this study are not wholly dependent upon such a change.

The blood levels of the constituents studied apparently return to normal within five days of an attack of parturient paresis. This previously has been shown to be the case for blood levels of calcium and citric acid (2).

It is well to recognize that the number of animals involved in this study is small, particularly the control groups. However, if the results presented here are substantiated by further research, additional clues to the etiology of parturient paresis may be provided.

SUMMARY

The blood levels of calcium, sodium, potassium, chlorides, pyruvic, lactic and citric acid and hematocrits were determined on eight milk fever cows just prior to treatment and on about the fifth, tenth, fifteenth and thirtieth day following treatment.

The average values for the various blood constituents obtained before treatment indicate (a) lower calcium, (b) lower sodium, (c) higher potassium, (d) higher chlorides, (e) much higher pyruvic acid, (f) higher lactic acid, (g) lower citric acid and (h) higher hematocrits than the levels found in samples taken five days or longer after treatment.

Controls consisted of three normally calving cows from which blood samples were taken on the day of calving and during the same periods following parturition as for the above group. Also, the same determinations were made.

The average values of the blood constituents obtained on the day of calving for the latter group indicated (a) lower calcium, (b) lower sodium, (c) higher potassium, (d) higher chlorides, (e) slightly higher pyruvic acid, (f) no change in lactic acid, (g) slightly lower citric acid and (h) no difference in hematocrits from levels found in samples taken five days or longer following parturition.

Not all individual cows responded in the manner indicated by the averages.

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EFFECT OF TYPE OF PROTEIN ON THE RESPONSE OF YOUNG DAIRY CALVES TO AUREOMYCIN WITH DATA ON THE INTESTINAL MICROFLORA[1]

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A survey of the literature has shown that the composition of the diet may have a marked influence on the response (growth, feed efficiency, scours) obtained from feeding an antibiotic to poultry (6, 10, 13, 17, 21, 25) and swine (2, 7, 14, 16, 27, 28). The diets used in calf experiments involving antibiotics have varied in the kind of protein source. Dried skimmilk and dried whey have been the chief sources of proteins in milk replacement diets (5, 15), while cottonseed meal and soybean oil meal have been used extensively in calf starters (3, 9, 18, 20, 22). It was decided to test whether the type of protein source in an all-vegetable calf starter would influence the effect of aureomycin on young calves when weaned from milk at an early age; also, to study any change in the normal intestinal microflora of calves receiving the antibiotic. Our previous study (22) on the rumen microflora of calves did not reveal any change in the usual microscopic appearance when aureomycin was fed. Studies on the effect of feeding antibiotics on the intestinal flora of chickens and turkeys (1, 11, 23) and pigs (8, 23, 26) have indicated some changes in the bacterial groups of the feces of these animals. However, no work has been reported on similar investigations in ruminants.

EXPERIMENTAL

Thirty-six purebred male calves, 18 Holsteins and 18 Jerseys of the Louisiana State University dairy herd, were divided into three comparable groups. Each group was fed a 19 per cent digestible vegetable protein calf starter containing either soybean oil meal, cottonseed meal, or degossypolized cottonseed meal[3] as the main protein source. The percentage composition of the calf starters is given in Table 1. One half of the animals in each group served as controls, and the other half received aureomycin supplementation. A soluble tablet containing 50 mg. of crystalline aureomycin HCl was added daily to the milk of each calf in this group, and the calf starters for this group were supplemented with a 1 per cent level of Aurofac A (1.8 g. aureomycin per pound).

All calves received colostrum for the first three days after birth. On the fourth day of age, the animals were placed in individual pens with wood shavings. They were fed whole milk from a nipple pail at the rate of 1 lb. per 10

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[3] The meals were supplied through the courtesy of the Buckeye Cotton Oil Co., Cincinnati, Ohio.

TABLE 1
Composition of the basal calf starters

Ingredient	%		
Soybean oil meal, solvent extracted (44%)	27	....	....
Cottonseed meal, hydraulic (41%)	....	37	....
Cottonseed meal, degossypolized (41%)	....	....	37
Yellow corn meal	30	20	20
Oats	30	30	30
Wheat bran	10	10	10
Steamed bonemeal	2	2	2
Salt	1	1	1

The starters contained 19.14-19.17% digestible protein, 76.5-70.7% TDN and 8.25-8.44% crude fiber.

lb. of body weight to 30 days of age. Calf starters were fed *ad libitum* from the eighth day of age until a maximum of 4 lb. was being consumed by the Jerseys and 5 lb. by the Holsteins. A medium grade lespedeza hay was fed on the eighth day and 120,000 I.U. of vitamin A were fed each calf daily after weaning. All calves were weighed at weekly intervals through 16 weeks of age. Daily observations of the feces and physical appearance were made for each calf. Statistical analysis of the data was made according to the method of Snedecor (24).

Samples of feces were removed from the rectums of the calves by means of a long handled spoon. One gram of the moist feces was weighed out and appropriate dilutions made in sterile water blanks. Moisture content of the feces was determined by drying a sample at 110° C. The groups of bacteria were determined as follows: Total aerobic counts were made on a peptone-tryptone-glucose-yeast extract medium. The total anaerobic counts were made on the same medium but the petri dishes were incubated in a Brewer anaerobic jar. Since lactic acid bacteria have been shown to constitute a part of the intestinal flora of some animals (1, 11, 23), the carrot-liver agar medium of Garey *et al.* (12) was used to obtain maximum growth of these organisms. Coliforms were determined on Violet Red Bile agar and confirmed in 2 per cent Brilliant Green Bile Broth. The Most Probable Number of enterococci was determined in Azide Dextrose Broth using five tubes of each of three dilutions. Hydrogen sulfide-producing anaerobes were enumerated on a sulfite-tryptone-iron medium incubated in a Brewer anaerobic jar. Black colonies from this medium were inoculated into iron-fortified milk to determine probable presence of *Clostridium perfringens* types. All media were incubated at 37° C., except the Azide Dextrose broth, which was incubated at 45.5° C., and the H_2S anaerobic medium, which was incubated at 44° C. All counts were calculated on a dry weight basis.

RESULTS AND DISCUSSION

Table 2 gives the average daily gains, feed efficiency and incidence of diarrhea for each group of calves at 16 weeks of age. Examination of this table shows that aureomycin supplementation, with the exception of the Jerseys in group II on the cottonseed meal starter, produced an increase in weight regard-

TABLE 2

Effect of aureomycin on the average growth rate, feed efficiency and incidence of diarrhea in dairy calves at 16 wk. of age

Group and ration		Average daily gains (lb.)			Lb. of starter/lb. gain			Av. incidence of scours
		Holstein	Jersey	Group	Holstein	Jersey	Group	
								(d)
I Soybean oil meal	Basal	1.33	.90	1.11	2.24	2.11	2.17	2
	Aureomycin	1.60	1.06	1.33*	1.99	2.10	2.04	0
II Cottonseed meal	Basal	1.36	.86	1.07	2.40	2.57	2.48	.2
	Aureomycin	1.50	.89	1.19*	2.26	2.59	2.42	.2
III Cottonseed meal degossypolized	Basal	1.24	.68	.96	2.60	3.06	2.84	.3
	Aureomycin	1.37	.89	1.13*	2.29	2.40	2.35	0

* $P > 0.05$.

less of the type of protein in the starter. The average daily gains for both Holsteins and Jerseys fed aureomycin was 10 to 20 per cent higher than the average for the basal groups. An analysis of variance of the data showed that these differences in growth rate were significant at $P = 0.05$. This growth-stimulating effect of aureomycin in dairy calves has been demonstrated by other investigators also (3, 5, 15, 18, 20, 22). The aureomycin-fed calves on the soybean oil meal starter showed the greatest increase in weight. From this investigation it appears that the type of protein source (soybean oil meal) in an all-vegetable calf starter when fed with limited milk may affect the growth response of the calf to aureomycin supplementation. Feeding aureomycin daily in the milk produced a slight weight advantage over the basal groups. However, most of the weight advantage appeared after the animals were weaned (30 days of age) and were consuming the supplemented calf starters.

The calves on the soybean oil meal (group I) consumed less starter per pound of gain than did the calves in the cottonseed meal groups. The aureomycin-fed Holstein calves in all groups and the aureomycin-fed Jersey calves in group III consumed less feed per pound of gain as compared to the basal groups. This reflects the improved feed efficiency due to aureomycin supplementation, as has been reported by other investigators (4, 19). The influence of breed also is noticeable in these results.

Very little scours was encountered with the exception of the calves on the basal soybean oil meal ration, and these instances were of very short duration. However, this did not appear to influence the growth rate of the calves. One aureomycin-fed Jersey calf on the cottonseed meal starter scoured. This was the only case of scours encountered among the antibiotic-fed calves. The degossypolized cottonseed meal did not appear to have any advantage over the hydraulic cottonseed meal.

Thus, calves weaned from milk at 30 days of age and fed an aureomycin supplemented calf starter containing soybean oil meal as the principal protein

showed an increased gain in weight and improved feed efficiency over calves fed the same calf starter with a source of cottonseed meal as the main protein.

Bacteriological. The ranges of the total counts obtained on 62 fecal samples are given in Table 3. The total aerobic, anaerobic and carrot-liver shake tube counts, as well as the coliform and enterococci counts, usually were lower from calves that were no longer receiving milk in their diet. No significant difference was found between the counts on feces from calves receiving aureomycin and those not receiving it. The carrot-liver medium always gave the highest counts, even though the organisms growing on it during the last weeks of the investigation were not lactics. Hydrogen-sulfide producing anaerobes of the *Clostridium perfringens* type were encountered occasionally but were not present in significant numbers in any of the samples.

In calves less than 4 weeks of age the amount of dry matter in the feces was usually around 18 to 22 per cent, whereas in older calves the content of dry matter usually was 21 to 30 per cent. In two cases where scours were evident the dry matter content dropped to 13 per cent and, in each case, all counts were considerably higher than in other samples not showing scours.

Although the calculation of all counts on a dry weight basis did decrease to some extent the variations between similar samples, wide variations in the counts from different calves on the same diet or from the same calves at different times still occurred. Even though the number of samples from any particular group of calves is small, in our opinion the data indicate that the suppression of total numbers of bacteria or of three special groups, namely, coliforms, enterococci, and *Clostridium perfringens* types, is not the mechanism whereby aureomycin stimulates the growth of dairy calves. Other groups of microorganisms or some mechanism other than microbial inhibition may be involved. Rosenberg *et al.* (21) have reported recently that high levels of terramycin did not affect the intestinal microflora of the chick although growth was stimulated. Since our previous study (22) gave no indication of aureomycin affecting the rumen microflora, it is possible that the mode of action of antibiotics in stimulating growth in calves may be concerned with a more rapid or complete absorption of nutrients passing through the gastro-intestinal tract, or that certain biochemical reactions are influenced, resulting in increased synthesis of essential nutrients without altering the character of the microflora.

SUMMARY

An aureomycin-supplemented vegetable calf starter containing solvent-extracted soybean oil meal as the main source of protein showed a better growth response and more improved feed efficiency than when it contained either hydraulic or degossypolized cottonseed meal. Aureomycin-fed calves showed a growth increase of 10 to 20 per cent over control calves at 16 weeks of age.

A bacteriological study of the fecal contents has indicated that the mode of action of aureomycin in stimulating calf growth apparently is not due to selective bacterial inhibition of the common bacterial groups of the intestines, namely, coliforms, enterococci, and *Clostridium perfringens* types. Other microorganisms or other mechanisms may be involved.

TABLE 3

The effect of aureomycin on different groups of bacteria in the feces of calves of various ages

Group and ration		Range of counts per gram of dry weight for:											
		Aerobic $\times 10^7$		Anaerobic $\times 10^7$		C-L shake tubes $\times 10^7$		Coliforms $\times 10^6$		Enterococci $\times 10^3$		H_2S-producers $\times 10^3$	
		1-5 wk.	6-12 wk.	1-5 wk.	6-12 wk.	1-5 wk.	6-12 wk.	1-5 wk.	6-12 wk.	1-5 wk.	6-12 wk.	1-5 wk.	6-12 wk.
I Soybean oil meal	Basal	(5)* 2-940	(4) 15-200	(3) 151-960	(2) 192-400	(5) 280-2600	(3) 8-1000	(4) 590-1700	(6) 4-2800	(5) 10-700	(4) 68-170	(2) <1	(3) <1-200
	Aureo.	(2) 6-15	(4) 4-130	(0)	(2) 24-127	(2) 150-200	(4) 7-350	(2) 56-190	(4) 4-810	(2) 3400-4000	(4) 10-780	(0)	(2) <1
II Cottonseed meal	Basal	(5) 1-1000	(6) 1-31	(3) 14-1700	(4) 20-500	(5) 310-14000	(4) 2-1200	(6) 24-2100	(7) 6-72	(5) 230-35000	(6) 2-38	(2) <1-500	(4) <1-180
	Aureo.	(5) 4-1100	(4) 3-160	(2) 41-1400	(4) 17-270	(5) 55-8900	(2) 73-310	(5) 36-1600	(5) 4-170	(5) 360-430000	(4) <1-2100	(2) <1-7	(2) <1-9
III Cottonseed meal goss.-free	Basal	(5) 13-360	(3) 20-28	(6) 48-1500	(3) 28-35	(6) 75-2600	(0)	(6) 51-1300	(2) 20-80	(7) 4-80000	(3) <1-18	(3) <1-25	(3) <1-3
	Aureo.	(6) 29-470	(3) 3-6	(6) 14-910	(1) 16	(6) 15-1300	(2) 2-5	(6) 35-1500	(3) 2-68	(6) 80-70000	(3) 1-216	(3) <1	(3) <1

*No. of animals.

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THE CAROTENE REQUIREMENTS FOR REPRODUCTION IN GUERNSEY CATTLE

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Earlier work by Kuhlman and Gallup (7) has shown that carotene intakes of 45 γ per pound body weight are required for Jersey cows for successful reproduction. Converse and Meigs (1) reported that the carotene requirements during the last months of the gestation period of dairy cattle were between 80 and 120 γ per pound body weight. The daily allowances recommended by the National Research Council (8) are 60 γ per pound body weight for maintenance with an additional daily allowance of 30 mg. per cow during the last 2 to 3 months of gestation period.

Working with beef cattle, Davis and Madsen (2) found satisfactory reproduction when the carotene intake was maintained at 60 γ per kilogram body weight. Guilbert *et al.* (5) observed successful reproduction with a carotene intake of 26 to 33 γ per kilogram body weight, provided this was increased five-fold during the last month of pregnancy.

Differences among these values are related in part to differences in breed, carotene source and methods of carotene assay.

This paper presents the results of an eight-year study relative to the carotene requirements for successful reproduction in Guernsey cows.

PROCEDURE

At the age of 6 months, Guernsey heifers were placed on a diet consisting of prairie hay, cottonseed meal, hominy feed and beet pulp. The amount of prairie hay fed was controlled on the basis of monthly analyses so that the daily carotene intake ranged from 30 to 150 γ per pound of body weight. The other feeds were fed in such amounts that the total ration satisfied Morrison's maximum recommendations. Minerals were supplied free choice in a dry exercise lot. The heifers were bred at the age of approximately 15 months. The cows were not rebred earlier than 85 days after the preceding parturition.

Observations were made on the number of services per conception, length of the gestation periods and the condition of the calves at birth. Monthly analyses were made for blood plasma carotene and for butterfat carotene during the lactation periods.

A 20-cow herd of registered Guernseys was maintained for this study. Although 31 females were involved in the herd during the entire period, two were lost as non-breeders and four through fatal illness prior to the first gestation, leaving 25 animals available for observation.

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[1] Deceased.

An attempt was made to assign the cows to the experimental groups in such a manner that they would complete at least two gestation periods at different levels of carotene intake. Five cows completed only one gestation period during the study. The distribution of the number of cows involved in each of the intake groups is indicated in Table 1.

Carotene in the hay was determined by saponification and phasic separation between petroleum ether and 90 per cent methanol as described elsewhere (3, 9). Plasma carotene was determined by Kimble's procedure (6) and butterfat carotene by total yellow color of a dilute solution in petroleum ether. An Evelyn photoelectric colorimeter equipped with a 440-mμ filter was used in this work after calibration with the proper blanks and known concentrations of carotene (4).

TABLE 1

The effect of the average daily carotene intake during gestation on reproductive efficiency of Guernsey cows

	γ Carotene/lb. body weight			
	30-59	60-89	90-119	120 and over
Cows:				
Average blood plasma carotene (*γ/100 ml.*)	313	337	454	430
Number of cows	12	18	12	4
Number of gestations	16	30	21	5
Normal[a]	12	20	19	4
Pre-term	4	5	1	1
Abortions	0	4	1	0
No record	0	1	0	0
Retained placenta	11	8	7	1
Average services/conception	1.8	2.5	2.7	1.9
Calves:				
Number produced	16	26	20	5
Normal	6	13	16	3
Blind	4	3	0	0
Dead at birth	1	2	2	0
Slightly weak	2	2	2	1
Very weak or lacking coordination	1	3	0	1
Eyes bulged, lacrimating, etc.	1	2	0	0
No record	1	1	0	0

[a] Normal, 270 days and over; pre-term, 210 to 270 days; abortions, less than 210 days.

RESULTS

A summary of the reproductive efficiency involving 72 gestation periods is presented in Table 1. The data are compared on the basis of four different carotene intake groups as follows: 30-59, 60-89, 90-119, 120 γ and over per pound body weight daily. The gestation periods were compared in three categories, the periods compared being the normal length arbitrarily selected as 270 days or over, pre-term births 210-270 days and abortions less than 210 days. One living calf was presented at 210 days, and this time was chosen as the dividing point between abortions and later premature births. The actual length of those gestation periods terminating in abortions ranged from 70 to 197 days. Apparently, none of the abortions were associated with brucellosis or trichomoniasis.

Early termination of gestation periods was most prevalent when the daily carotene intake was less than 90 γ per pound body weight. Not including the abortions, the average length of gestation period observed in the four intake groups compared was 272, 275, 281, and 277 days, respectively. The average length of all gestation periods including those terminating in abortion was 272, 260, 274, and 277. Only five abortions were experienced in the 72 gestation periods and four of those occurred in animals whose daily carotene intake ranged from 60 to 89 γ per pound of body weight. The most significant abnormality relative to parturition was a high incidence of retained placentae encountered in the 30- to 59-γ intake group.

Only 45 per cent of the calves produced by cows receiving less than 90 γ carotene per pound body weight were normal at birth. When the daily carotene intake was above this level, 76 per cent of the calves were normal at birth. The abnormalities were much more severe among the calves produced by the low-carotene intake cows than among those produced in the high-intake groups. The birth of blind calves and of calves with other eye abnormalities was confined to the low-carotene groups. The highest level of carotene intake at which congenital blindness occurred was 85 γ per pound of body weight daily.

The average services per conception for the entire group of animals in this study was 2.3. There was no apparent relationship between conception and the carotene intake during the service period. The average services per conception for each of the intake groups were 1.8, 2.5, 2.7, and 1.9, respectively.

The average blood plasma carotene tended to vary in relation to the level of intake; however, the correlation was very low ($r = 0.36$). The large degree of individual variation in blood plasma levels as related to carotene intake can be demonstrated by citing examples from a scatter diagram on which intake was plotted against blood plasma levels. Five individuals with an average daily carotene intake of 70 γ per pound body weight maintained blood plasma carotene levels ranging from 200 to 945 γ per 100 ml. Nine other individuals that maintained blood plasma carotene levels of 350 ± 11 γ per 100 ml. had average daily carotene intakes which varied from 37 to 141 γ per pound of body weight.

Similar results were obtained with respect to the butterfat carotene. Six individuals receiving daily carotene intakes ranging from 53 to 145 γ per pound body weight maintained butterfat carotene outputs between 2.25 and 2.50 γ per gram of fat. The range of butterfat carotene for all the animals in the trial was 1.55 to 4.65 γ per gram.

DISCUSSION

It appears that the minimum carotene requirements for successful reproduction in Guernsey cattle may be somewhat higher than for some other breeds. Considerably more individual variation was observed in the study of this breed as compared to a similar study with Jersey cattle. In a few instances, normal gestation periods terminated with the presentation of normal calves after very low levels of carotene intake. It should be emphasized, however, that one blind calf was produced by a dam receiving 85 γ carotene per pound body weight

daily. On the basis of the results of this study, it appears that a daily carotene intake of 90 γ per pound body weight is the minimum safe level to insure normal reproduction in Guernsey cattle.

This trial was so designed that the carotene intake was maintained at a definite level for the entire gestation period. Further work is being carried on to study the efficiency of reproduction in cows receiving a maintenance level of carotene during the first portion of the gestation period when followed by an intake of 90 γ carotene per pound body weight during the last 90 days. The present data, however, give indications that a relatively high carotene intake during the entire gestation period is desirable. It was noted that abortions usually occur prior to 180 days. If it is correct to associate certain abortions with critical intake levels of carotene, it appears that minimum carotene requirements for reproduction should be considered for a longer period than only the last 90 days of gestation.

SUMMARY

The efficiency of reproduction as related to carotene intake has been studied for a period of eight years with a small herd of Guernsey cows. The sole source of carotene in the cows' rations was prairie hay which was fed in such amounts that the daily carotene intake varied between individuals from 30 to 150 γ per pound of body weight during the entire gestation period. Seventy-two gestation periods were observed in the study.

From the results of this trial it appears that the minimum safe level of carotene intake for successful reproduction in Guernsey cattle is 90 γ per pound of body weight daily. In order to decrease the hazard of abortions and on the basis of other indications which are now under study, it appears desirable to insure this level of intake during the entire gestation period. No definite relationship appeared to exist between the level of carotene intake and the conception rate as measured by services per conception.

There was a low correlation between carotene intake and carotene blood plasma levels and butterfat carotene. Because of the large variation in blood plasma and butterfat carotene between individuals in this study, these criteria did not appear to be reliable as measurements of adequate carotene intake.

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TESTING DAIRY BULLS UNDER THE PENKEEPING SYSTEM IN BRAZIL[1]

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One of the prevailing systems of dairy cattle management in the dairy region of Minas Gerais is the so-called penkeeping system or *sistema de retiros*. Briefly, the general management is as follows: The calves are with the cows during the day and are shut away from them at night. The cows are milked only once a day. This is in the morning before the calves are turned in with the cows for the day. The amount of milk is recorded twice a month. During the rainy season the cows have only pasture and salt, but in the dry season some grass silage and chopped sugar cane and cottonseed meal are fed. All cattle are dipped twice a month for ticks.

Some factors which affect milk yield under this type of environment have been identified and described already (1, 2, 3, 8). Length of the lactation period varied widely and accounted for 47.4 per cent of the variance in the actual milk yields in the complete lactation. Consequently, factors for correcting to a standard 305-day length were developed. In these 305-day corrected milk yields, the permanent differences between cows caused 44 per cent of the variance. None of the other factors investigated had an important influence on milk yield.

The use of grade bulls in the tropics has been reported by several investigators. Smith (10) has outlined the principal methods of improving cattle in the tropics as follows: (a) by introducing exogenous breeds into countries where no native cattle existed before or by replacing the native cattle by the imported breeds; (b) by using purebred bulls on native cows enough generations to reach what is called the *puro por cruza* stage, *i.e.*, about five or more top-crosses with purebred sires; (c) by using purebred bulls fewer generations so as to introduce certain desirable qualities into the native cattle but without losing all of the desirable qualities which the native cattle have; (d) by selecting native cattle for milk production.

Edwards (4), studying cattle of Jamaica, has emphasized the value of backcrossing high-grade cattle to the native stock as a means of increasing milk yield. He discussed the possibility of developing a new breed suited to the tropical conditions by mating together individuals possessing $\frac{1}{32}$ to $\frac{1}{4}$ Zebu blood.

Howe (5), also in Jamaica, found that the half-breeds of Zebu and Holstein, Jersey or Guernsey produced more milk than any other grades or purebreds. However, his data did not permit him to separate the effect of heterosis

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[1] The writer is indebted to Dr. Jay L. Lush for guidance and assistance during the course of this study.

from that of percentage of blood and he did not have higher percentages of Zebu blood to carry the analysis further.

In Brazil, Rhoad (8) reported the extensive use of grade bulls in the commercial herds. He believed that the general use of a method close to the "Method C" of Smith was rapidly forming a new kind or strain of dairy cattle in the State of Minas Gerais.

The primary object of the present study was to measure the repeatability and heritability of differences between cows and to see whether there is any significant difference between daughters of several bulls. Whether the purebred bulls were better than the grade ones in the same herd also was studied. The present study was a pilot one and is expected to be followed by more research.

SOURCE OF DATA AND METHODS

The data for the present analysis were made available by O. B. Junqueira, Leopoldina, Minas Gerais. The cattle are mostly grades with various amounts of Simmenthaler blood. Only lactation records which appeared normal were used in this study. The records discarded as abnormal included those with known occurrence of foot and mouth disease, death of the calf, records initiated by abortion and records of cows raising two calves. All records were corrected to a standard length of 305 days in accordance with the correction factors developed previously (1).

For the present analysis, 300 daughter-dam pairs were available. The daughters were by 27 sires which had various fractions of Simmenthaler blood (alone or in combination with Guernsey or Swiss, or both) and one purebred Holstein bull of Dutch breeding which had 10 daughters. These bulls were not entirely contemporary but they were all treated as a single sample since the calendar year in which the cows calved showed only a slight influence on milk yield (2).

In studying the regression of daughter on dam, each cow that had more than one daughter was used in the analysis as many times as she had daughters. The production figures for each dam and for each daughter were averages of all the normal lactation records available for each. As the present work was planned to study repeatability also, the mates of a sire were included only if they had at least two records. All daughters were included, even if they had only one record.

Most of the results are given in terms of analysis of variance and covariance as described by Snedecor (11).

RESULTS

Repeatability of milk yield. Repeatability of milk yield measures the average degree to which a cow will produce in her next lactation as much above or below the average of the herd as she did in the lactation already made. The intra-herd repeatability of single records usually is of the order of 0.4 in many different kinds of data. Under the penkeeping conditions a correlation of 0.44 has been found (1) between two consecutive lactation records. Although the

present study deals with part of the same data, the repeatability was computed on the whole of the present data in order to reach greater accuracy.

Repeatability was determined here by placing the cows mated to each sire into a high or low half according to their milk production in their first 305-day lactation record. The later records of each cow were averaged into a single figure. Only even numbers of mates were used. If the number was odd, the mate whose first record was the median of the group was discarded. The repeatability r between single records is:

$$\frac{(\text{Average later records of high mates}) - (\text{Average later records of low mates})}{(\text{First record of high mates}) - (\text{First record of low mates})}$$

This closely approximates the regression of later records on first record.

The repeatability found in this way was 0.41, which is similar to the 0.44 (1) and 0.43 (6) reported previously. Although the conditions of management considered here are very different from those prevailing in the sources of the data used by Lush *et al.* (6), the repeatabliity is about the same in both cases.

Daughter-dam comparisons. The number of daughter-dam comparisons per sire ranged from 5 to 29, the average being 11.2. The average number of lactations per dam was 5.7 $\pm$ 0.12 and per daughter was 3.4 $\pm$ 0.13. The differences of 2.3 lactation records is highly significant statistically ($P<0.01$), but it probably is without any biological or practical significance because it results automatically, at least in part, from the fact that in order to be included, a dam had to have at least two records and also a daughter with a record, while all daughters which had even one normal lactation record were used. Also, more of the daughters would not yet have finished their lifetime production at the time the study was made.

The average milk production per 305-day lactation period was 1,209 l. for the dams and 1,140 l. for their daughters. The difference of 69 l. is highly significant ($P<0.01$). Only six of the 27 sires tested had daughters which averaged more than their dams. But this comparison is not wholly fair because the dams were more highly selected. In the first place, dams culled before they had completed a second normal lactation record are not included. Also, the dams with more daughters are included more often, whereas a daughter is included only once. How to measure and discount this bias is not apparent, but clearly it is in the direction of making the sires appear poorer than they really were.

Differences between sires. The analysis of variance and covariance among records of dams and daughters is seen in Table 1. The differences between the groups were significant for the dams and highly significant for the daughters. Let A stand for the variance component due to the real differences between the averages of the s groups and B for the variance component between the k individuals within a group. Then, if a cow is compared with all the others in turn, $k-1$ of the differences will be between cows in the same group, while $k(s-1)$ of the differences will be between two cows belonging to different groups. Half the mean square difference between cows belonging to the same group will be B.

TABLE 1

Analysis of variance and covariance among records of dams and daughters

Source of variation	Degrees of freedom	Daughters(Y) mean square	ΣXY	Dams(X) mean square
Total	299	79,325	2,833,134	45,529
Between sires	26	234,292	−59,222	76,848
Within sires	273	64,567	2,892,356	42,546
		B = 64,567		B′ = 42,546
		A = 15,290		A′ = 3,090

Half the mean square difference between cows belonging to different groups will equal $A + B$. The mean square between groups will be $kA + B$.

The time elapsed between the use of various bulls may have permitted environmental circumstances to change enough to contribute a little to A' and distinctly more to A, since the daughters' records were more nearly contemporary than the records of the dams. Each group of dams probably included a few half-sisters, these being daughters of the preceding sires. This would contribute a little to A'. All of the daughters were paternal sisters of course, and this would contribute a quarter of the additively genetic variance to A. A also would contain a little because some of the dams would be related. In terms of A and B, the correlation between paternal sisters was $A/(A + B) = 0.19$. Between cows mated to the same bull, it was $A'/(A' + B') = 0.07$. Because both of these (especially the former) may contain some environmental contributions, they do not seem suitable evidence for estimating heritability.

Heritability of milk yield. Heritability can be defined as $G/(G + E)$ where G is the variance due to hereditary differences between individuals and E is the rest of the variance between individuals. In the present analysis, the intra-sire regression of daughters on dams was 0.2125 but, since the data are lifetime averages, doubling this regression will estimate the heritability of differences between those lifetime averages, rather than differences between single records. The averaging tends to leave in the average of n records only $1/n$ of that variance W due to things which vary randomly from one record to another from the same cow. The observed regression is $\frac{1}{2} \cdot \frac{G}{C + W/n}$ where C is the cow component, W is the rest of the variance in single records and n is the average number of records (4.5). Repeatability of single records is $\frac{C}{C + W}$. Using 0.4 for that, we find 0.23 for $\frac{G}{G + E}$; that is, for the heritability of differences between single records. Sampling errors would make the 95 per cent confidence interval extend from about 0.10 to about 0.36. Besides this purely sampling error, it is conceivable that this regression contained something from environmental correlations between daughter and dam, although this is unlikely because the regression was computed on an intra-sire basis and there was no intention or conscious effort by the manager to make the environmental variations alike

for daughter and dam. The estimate of 0.23 for heritability does agree well with other published values, but it certainly cannot yet be considered as established within narrow limits.

Sires' indexes. Estimating the sires' transmitting abilities under the pen-keeping system is important, since there is much controversy about the use of purebred and grade bulls in the several types of herds in the dairy regions of Minas Gerais. The various types of indexes of dairy bulls which have been proposed differ mainly in their variability and in how much use they make of the records of the dams of the daughters.

Here it was thought worth while to try to discount for the probable effects of selection among the dams by estimating each dam's breeding value *G* from the formula (6):

$$G = A + \frac{nh\,(D - A)}{1 - r + nr}$$

where A is the average 305-day corrected yield of the herd in which the dams were tested (A was taken to be 1,165 l. in the present case).

n is the number of lactation records for that cow.

D is the average of these *n* records.

h is the heritability of milk production found here (0.23).

r is the intra-herd repeatability of single records (0.40).

The index used for each sire was: twice his daughter average, minus the average *G* for his mates. This is the same as the equal-parent index, except that the records of the dams (but not those of the daughters) were regressed toward the breed average to allow for selection and imperfect repeatability and heritability.[2] With a little over five records per dam, this index is nearly the same as Rice's "regression index," except that this one is more variable because the daughters receive double weight.

The indexes ranged from 585 to 1,717 l. They probably still include considerable variance from chance at Mendelian segregation and some from environmental effects, especially on the daughters since their records were used without regression and often were nearly contemporary.

The sires were sorted in five different ways, thought worth studying: (a) they were placed in 17 groups by the amount of imported blood that they had; (b) by their total fraction of imported blood, no matter from which breed; (c) purebred (Simmenthaler and Holstein) *versus* grade sires of any amount of blood of any breed; (d) purebred Simmenthalers *versus* grade Simmenthalers (no other blood included); (e) among grade sires, comparison between groups with only Simmenthaler blood and those having Simmenthaler and Guernsey, or Simmenthaler and Swiss, or Simmenthaler and Guernsey and Swiss. In none

[2] The increase in accuracy thus gained may have been too small to have been worth the arithmetical work, although this still seems uncertain.

of these groupings were the differences between groups significant, although in two cases the F value was close to the 0.05 limit.

The averages of the indexes are:

Grouping b:

Fraction of imported blood	No. of sires	Av. index (liters)
8/8	5	1,017
7/8	2	684
6/8	7	1,022
5/8	5	878
4/8	7	1,186
3/8	1	910

Grouping e:

	No.	Av. index
Only Simmenthaler + native	9	1,103
Simmenthaler + Guernsey + native	5	953
Simmenthaler + Swiss + native	5	1,025
Simmenthaler + Guernsey + Swiss + native	2	615

The five purebreds averaged 1,017 and 22 grades averaged 1,006 l.

The differences among the indexes were not consistent, and the numbers were few. The subject needs further investigation, since there was a hint that the value of the indexes decreased as the amount of imported blood increased, except for the few purebred bulls. The higher indexes of the purebred bulls might be due to heterosis, or possibly to better care given their calves because so much had been invested in the sires, although this could not be tested here. The high cost of imported sires probably was only a minor reason for many of these sires not having more imported blood. The other reasons include a wish to maintain or reestablish adaptability to the environment. Also, bulls with higher fractions of Simmenthaler blood were scarce.

SUMMARY

Grade dairy sires have been used extensively in the dairy regions of Minas Gerais, Brazil, where milk is produced under the penkeeping system. Data from one of these herds showed 0.41 for the repeatability of single records and 0.23 for the heritability of differences in single records of milk yield. These values are similar to those reported in the temperate regions under a widely different kind of management. Variance within and between the 27 sires indicated a correlation of 0.19 between paternal half sisters but may have contained something from common environment and time trends.

Variation in the sire indexes did not seem related to the fraction of imported blood. Individual genotypic differences seemed more important than the amount of exogenous blood.

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FURTHER STUDIES OF CAVITATION IN THE HOMOGENIZATION OF MILK PRODUCTS

C. C. LOO[1] AND WALTER M. CARLETON[2]

Homogenization has been experiencing an increasing application in the milk industry since its discovery half a century ago. Several theories, without substantial experimental support, have been advanced to explain the mechanism of homogenization. Among them the more popular ones are shearing, grinding, exploding, and impinging. In 1949, Loo *et al.* (7) concluded from their experiments that homogenization was due partially to a physical phenomenon known as cavitation. Cavitation, the rapid formation and subsequent collapse of bubbles in a liquid, has been known for some time as the cause of certain kinds of damage in ship propellers and other hydraulic equipment. It also has been believed to have an important role in the production of emulsion by ultrasonic means.

It is believed that conventional homogenizers spend a great portion of the power supplied in overcoming friction, and, therefore, it appears worthwhile to investigate the possibility of more efficient homogenization in the light of cavitation effect.

Publications concerning homogenization of milk products have been plentiful. A voluminous and comprehensive bibliography on milk homogenization was prepared by Trout (11). However, the amount of literature concerning the physical process of homogenization has been small. A paper suggesting cavitation as a factor of homogenization was published by Loo, Slatter and Powell (7). Wenrich (13), in discussing the mechanism of homogenization, postulated that there should be a partial vacuum at the homogenizer valve. His postulation seems to be consistent with the cavitation theory of Loo *et al.*, because under usual conditions a partial vacuum is a necessary condition of cavitation.

The usual concept of cavitation procedure is that, when the hydrostatic pressure drops to vapor pressure, there will appear in a liquid vapor cavities which will collapse instantaneously when the vapor is forced to condense by rising hydrostatic pressure. A quite different theory was advanced by Van Iterson (12) in 1936, and later checked by Bottomley (3) theoretically and experimentally. They suggested that destructive cavitation bubbles were microscopic air bubbles in an unstable equilibrium state of supersaturation and that the hammering force, which might amount to 120 tons per square inch, was due to the release of surface energy on the collapse of the bubbles.

EXPERIMENTAL

The experimental work generally could be divided into two parts, namely, a critical examination of the theory of cavitation as a factor of homogenization

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and the design of a device for producing cavitation in milk products, together with a study of the homogenization characteristics of this device.

Part I. A Critical Examination of the Theory of Cavitation as a Factor of Homogenization

METHODS

Two ways of approach to examine the importance of cavitation in homogenization were tried. The first way was to keep constant all the factors, such as shearing and impinging, and to vary only the conditions of cavitation. This was achieved satisfactorily by keeping the homogenizer valve opening constant and varying the back pressure by means of a second-stage valve. The back pressure was expected to have an influence on the hydrostatic pressure at the vena contracta[3] at the homogenizer valve. To avoid the effect of additional variable shearing and impinging at the second-stage valve, milk samples were collected from between the homogenizer valve (the first-stage valve) and the second-stage valve by means of a steel cylinder inserted there, as shown in Figure 1. Control samples were obtained using no back pressure. The samples

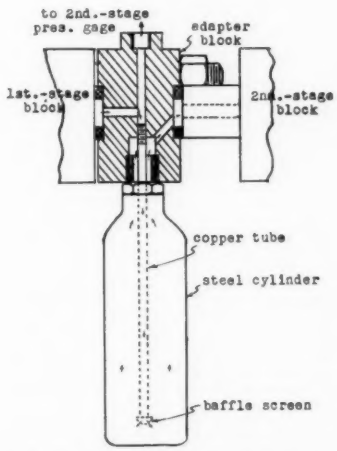

Fig. 1. Inserting a steel cylinder between the first-stage and the second-stage of a homogenizer.

were tested for degree of homogenization according to the United States Public Health Service (U.S.P.H.S.) definition of homogenized milk.

Theoretically, the back pressure should be reflected in the pump pressure by the same amount. However, it was found that this was not true in the case of the particular homogenizer used (a single-piston 25 gph machine). This

[3] Vena contracta is the contraction of flow lines of a liquid entering a sudden constriction.

might mean that the back pressure had an effect on the energy loss at the homogenizer valve and a constant pressure difference did not seem to be a reliable indication of constant valve opening. In order to overcome the complexity arising from this peculiar behavior of the homogenizer, two methods were tried for indicating a constant valve opening. One was the pressure difference across the valve; the other was the valve handwheel position. Separate gages were used to measure the back pressure and the pump pressure.

The other way of examining the importance of cavitation in homogenization was to investigate the pattern of pressure variation and the progress of homogenization between the valve surfaces. To do this, two homogenizer valves with a number of "bleeding holes" at different radial locations were used. A "bleeding valve" is shown diagrammatically in Figure 2. Plastic tubings con-

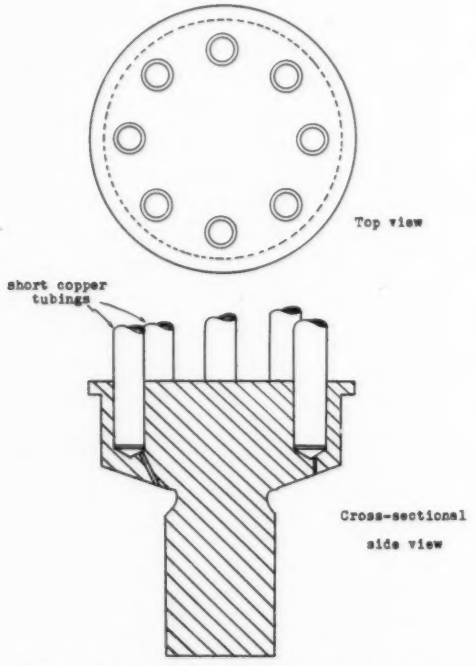

FIG. 2. Enlarged views of the "bleeding valve."

necting to the holes served as a means for collecting samples and for measuring the pressures. The heights of jets issuing from the tubings, when water was being pumped through the machine, were used to represent pressures at the respective holes. Since the quantity of milk coming out from each bleeding hole was not very great during a convenient length of time, the degree of homogenization was judged by the microscopic method devised by Farrall *et al.* (5).

RESULTS

Results presented in Tables 1 and 2 concerning the effect of back pressure on homogenization indicate that all the back pressures used reduced the efficiency of the valve.

TABLE 1

The effect of back pressure on the degree of homogenization of milk[a] when the pressure difference across the homogenizer valve was constant

Homo. pump press. (psi)	2,500	3,000	3,500	4,000
Back press. (psi)	0 (control)	500	1,000	1,500
Homo. pump press. on the release of back press. (psi)	2,500	2,850	3,200	3,600
Av. U.S.P.H.S. indices of 3 runs	8.3	14.0	13.4	9.3

[a] Temp. of the milk during homogenization was between 140 and 145° F.

TABLE 2

The effect of back pressure on the degree of homogenization of milk[a] when the homogenizer valve handwheel was at constant setting[b]

Homo. pump press. (psi)	2,500	2,650	2,750	2,900
Back pressure (psi)	0	500	1,000	1,500
Av. U.S.P.H.S. indices of 4 runs	10.5	13.1	15.8	21.2

[a] Temp. of the milk during homogenization was between 140-145° F.
[b] The homogenizer valve handwheel setting was such that it would give a pump pressure of 2,500 psi on the release of back pressure.

The pattern of pressure variation along the homogenizer valve clearance is shown graphically in Figure 3. It can be seen that the pressure experienced a sudden dip to almost atmospheric at a point between 0.01 and 0.02 in. from the inner edge of the valve.

Results presented in Table 3 reveal that the degrees of homogenization of milk samples collected from the hole 0.016 in. from the inner edge of the valve and the other outer holes were, as was shown by statistical analysis, all insignificantly different from that of the milk samples collected at the outlet of the homogenizer. In other words, homogenization was virtually completed at about

TABLE 3

Degree of homogenization (expressed in Farrall's index) of milk samples collected at various radial positions of homogenizer valves

Valve A

Distance from inner edge of valve (in.)	.008	.010	.013	.016	At homo. outlet
Av.[a] of 8 counts of Farrall's index	71.07	26.12	41.95	5.27	9.52

Valve B

Distance from inner edge of valve (in.)	.022	.045	.067	.089	.112	.134	.157	.179	At homo. outlet
Av.[b] of 8 counts of Farrall's index	24.7	16.5	19.1	23.8	33.1	27.8	15.1	[c]	24.8

[a] Least significant difference = 14.7 at 5% level and 19.8 at 1% level.
[b] No significant difference was disclosed by statistical analysis.
[c] No sample was obtained from this hole because of the negative pressure at this hole.

0.016 in. from the inner edge of the valve. Statistical analysis of the results was necessary to judge their significance, because the microscopic method almost always involves considerable variation in the readings even of identical samples.

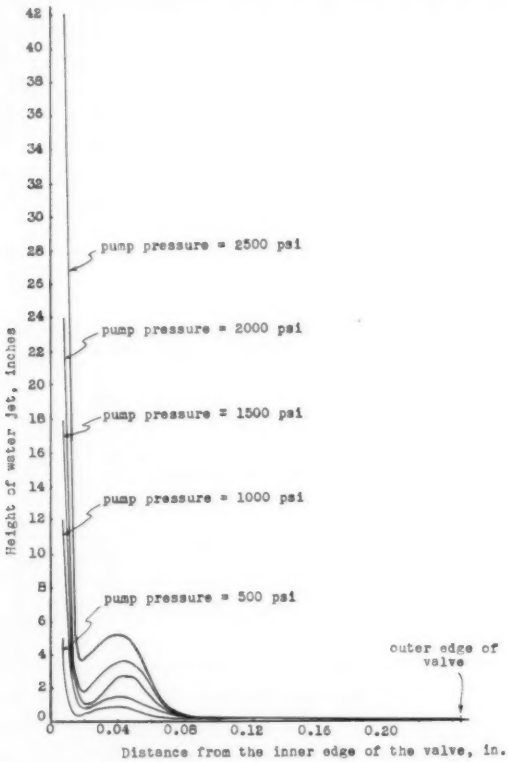
Height of water jet, inches
pump pressure = 2500 psi
pump pressure = 2000 psi
pump pressure = 1500 psi
pump pressure = 1000 psi
pump pressure = 500 psi
outer edge of valve
Distance from the inner edge of the valve, in.

FIG. 3. Variation of the hydrostatic pressure along the clearance of a homogenizer valve. The chart was constructed with the assumption that pressure was proportional to the height of the water jet issuing from a bleeding hole in the valve.

PART II. THE DEVELOPMENT OF A KNIFE-EDGE HOMOGENIZER VALVE AND ITS HOMOGENIZATION CHARACTERISTICS

The "bleeding valve" experiments showed that reduction in fat globule size was virtually completed at 0.016 in. from the inner periphery of the valve surface. Since the homogenizing region is so close to the inner periphery, one might think that that part of the valve surface beyond the homogenizing region has little contribution to the process of homogenization. On the basis of shearing theory, this hypothesis would appear justified. On the other hand, in the light of cavitation theory, the surfaces beyond the homogenizing region should cause a back pressure which very likely has an important influence on the intensity and quality of cavitation at the homogenizing region.

If the valve surface of a homogenizer valve is reduced to a knife edge, a great deal of the flow resistance may be removed. Also, the knife edge forms a sharp and sudden constriction to the flow and should aid materially the production of cavitation phenomenon. Thus, the knife-edged valve becomes a device that will generate cavitation more easily, and at the same time, through the use of a back pressure valve, the collapse pressure on the cavities can be independently regulated to any positive value. Such an independent regulation of the collapse pressure on the cavities is not attainable in a wide-surfaced valve, because the resistance due to the wide surface constantly backs up a high collapse pressure.

APPARATUS AND METHOD

Based on the preceding theory, a knife-edged homogenizer valve and a valve for controlling the collapse pressure (the exit side pressure, or the back pressure) were designed and are shown in Figures 4 and 5, respectively. The valve for controlling the back pressure consisted essentially of two rubber rings pressed together to form a variable smooth constriction.

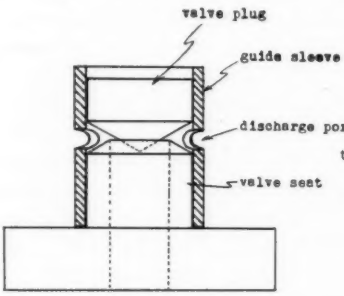

FIG. 4. Knife-edged homogenizer valve.

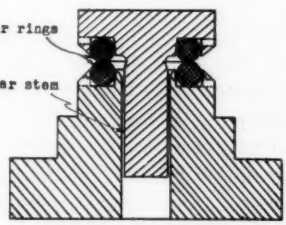

FIG. 5. Cavity collapse pressure control valve for use in connection with the knife-edged homogenizer valve.

A Vickers 9-piston hydraulic pump was used to generate smooth and steady pressure. Its capacity was adjusted to deliver 25 gal. per hour. The pump and back pressures were measured by separate gages. Since the amount of dissolved gases has been pointed out by several investigators (1, 2, 6, 9, 10) to be a very important factor in the production of emulsion by ultrasonic vibration, an attempt was made to study the effect of the amount of dissolved gases on homogenization efficiency. An air vent with a control valve was built into the suction side of the pump so that various amounts of air might be added to the milk product being homogenized and the effects due to the addition of air might be studied. When the air-liquid mixture came into the high-pressure side of the pump, the air would dissolve into the liquid unless its amount exceeded the amount necessary for saturating the liquid under the pump pressure.

The essential feature of this experimental homogenizer may be summed up as follows: (a) Its pressure was more uniform. (b) It was more easily possible

to cause cavitation. (c) It allowed the control of the collapse pressure of the cavities. (d) It allowed dissolving a controlled amount of air into the milk product being homogenized.

This experimental homogenizer was used to homogenize milk, concentrated milk and ice cream mix at various combinations of pump pressure, back pressure and air saturation pressure. The method of regulating the amount of air is described below.

Let P_1, P_a and P_2 be the desired pump pressure, air saturation pressure and back pressure, respectively. The air vent first was closed and the machine started. After the homogenizer valve (the first-stage valve) was adjusted to obtain P_1 on the pump pressure gage and the back pressure valve was adjusted to obtain P_a on the back pressure gage, the air vent valve was opened up gradually until a distinct rattling noise could be heard. At the same time, the pointer of the pump pressure gage began to show erratic vibration. Then the vent valve was turned back just far enough to reduce the rattling to a smooth hissing noise and the gage pointers just ceased erratic vibration. The back pressure valve then was tightened to get P_2 on back pressure gage. The procedure of obtaining the three pressures was then completed. After a reasonable length of time, a sample could be collected at the outlet of the homogenizer.

Milk samples were tested for the degree of homogenization according to the U.S.P.H.S. definition of homogenized milk. Concentrated milk was tested for degree of homogenization by Farrall's method and also for heat stability according to the method of Cole and Tarassuk (4). Samples of the same batch of concentrated milk homogenized by a Manton-Gaulin single piston homogenizer at 2,500 psi were used for comparison. Ice cream mix was tested for degree of homogenization by Farrall's method and for viscosity by means of a Brookfield viscosimeter. Samples of the same batch of mix homogenized by a Cherry-Burrell Superhomo at 2,300 psi pump pressure and 500 psi second-stage pressure were used for comparison.

RESULTS

Results plotted in Figures 6 and 7 show that there existed an optimum back pressure of 50 to 150 psi for the knife-edged valve. Milk could be satisfactorily homogenized at a pump pressure of 800 to 1,000 psi and a back pressure of 50 to 150 psi. Results showed that the addition of a small amount of air into milk had no significant effect on the homogenization efficiency but large amounts of air tended to reduce the efficiency.

It was found that the knife-edged valve operating at 1,000 psi pump pressure and 100 psi back pressure produced slightly better fat globule size reduction in concentrated milk than the Manton-Gaulin homogenizer operating at 2,500 psi. Heat stability was practically unchanged by either machine. Deaerated concentrated milk was less homogenized than non-deaerated samples. The addition of air up to 75 psi air saturation pressure had no significant effect on the homogenization efficiency.

Results of homogenizing ice cream mixes showed that the knife-edged valve operating at 1,000 psi pump pressure, 100 psi back pressure and 75 psi air

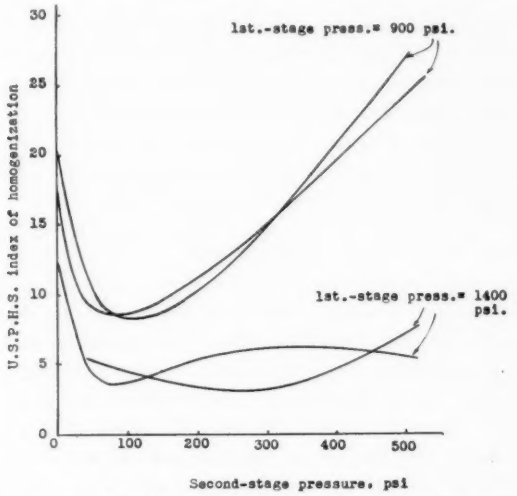

FIG. 6. Operation chart of the knife-edged valve for 0 to 500 psi second-stage pressure with no air injection.

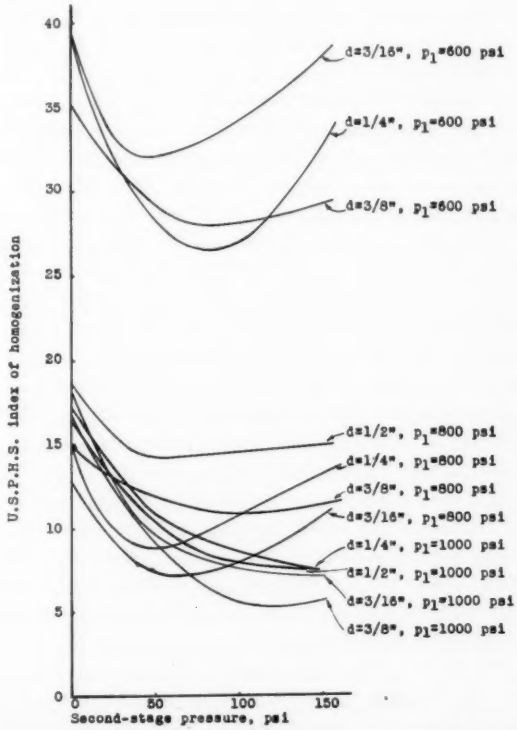

FIG. 7. Operation chart of the knife-edged valve for 0 to 150 psi second-stage pressure with no air injection.

saturation pressure could homogenize ice cream mix as well as a Cherry-Burrell Superhomo operating at 2,300 psi pump pressure and 500 psi second-stage pressure. With no addition of air, the ice cream mix was not as well homogenized even when the pump pressure was raised to 1,400 psi. Viscosities of the samples showing satisfactory fat globule size reduction were much lower when homogenized by the knife-edged valve, being at the order of 20 centipoises as compared with the Superhomo samples at about 70 centipoises.

DISCUSSION

It is evident from the experimental results that back pressure has some definite influence on the homogenization efficiency. In the case of the higher magnitudes of back pressure used the homogenization efficiency was lowered. The application of a back pressure should not affect the factors of shearing and impingement, because the homogenizer valve clearance was maintained constant. On the other hand, since cavitation is a result of lowering of pressure, the back pressure undoubtedly affects the intensity of cavitation. Hence, the reduction of homogenization by certain levels of back pressure was a very likely evidence of cavitation being a factor in homogenization.

The results of the bleeding valve experiments also yield evidence that cavitation occurs and is a responsible factor in homogenization of milk. The sudden dip in pressure to almost atmospheric at the third hole of the bleeding valve was a good indication of the occurrence of cavitation there. Though the pressure at that hole did not go below atmospheric, the true pressure at the vena contracta could have been below atmospheric, because the hole (0.0156 in. in diameter) possibly was several times larger than the distance between the top and the bottom halves of the valve and undoubtedly covered a radial distance greater than the contracted part of flow at the vena contracta. Therefore, it did not measure the pressure at the very neck of the vena contracta but measured the gross pressure of a region around the vena contracta.

The microscopic examination of the milk samples from the bleeding holes revealed that only those samples coming from the three innermost holes were significantly different, in degree of homogenization, from the milk collected at the outlet of the homogenizer. Since cavitation was likely to occur at about the third hole, as was manifested by the sharp dip in pressure there, the high degree of homogenization of milk samples coming out from the fourth and the following holes strongly indicated that cavitation was an important responsible factor of homogenization. Of course, there existed also the effect of shearing due to the bleeding holes themselves. Shearing due to the hole itself should be maximum at the innermost hole where the milk passed through it at a speed higher than at any of the other holes. Yet the lowest degree of homogenization was found in the milk from this hole. Therefore, it seems reasonable that the factor of shearing due to the holes could be neglected.

It was observed that a negative pressure existed near the outer edge of the valve when milk was pumped through it (see Table 3). The lowering in pressure here probably was the same as that happening in a radial flow between

any two parallel discs under ordinary conditions. This drop in pressure indicated that gaseous cavities could occur near the outer edge of the valve also. A cavity that grows so large as to include several globules in its shell may bring the globules together on its collapse. If the conditions are such that the bubbles collapse under dynamical equilibrium [such as the collapse due in vapor condensation under equilibrium conditions as hypothesized by Bottomley (3)] the globules might have the chance to agglomerate. If cavitation of this kind occurs at the negative pressure region near the outer edge of the valve, globules that have just been broken up near the inner edge would have the chance to form clumps.

The advantageous effect of a second-stage valve in the prevention of clumping in a conventional homogenizer might be due to its eliminating the negative pressure near the outer edge of the homogenizer valve. Of course, when a second-stage pressure of 500 psi or higher is used, there could be some homogenizing effect in the second-stage valve itself also.

The phenomenon of optimum back pressure for homogenization by means of the knife-edged valve hardly can be explained on the basis of shearing and impingement. However, in the light of cavitation theory, a satisfactory explanation is that the optimum back pressure is just high enough that it intensifies the striking effect of the collapsing cavities, yet it does not cause any suppressing effect on the generation of the cavities.

The operation chart of the knife-edged valve (Figure 6) probably can be applied to the conventional poppet-type homogenizer valves, too. However, because of the constant high collapse pressure due to wide valve surface, the operation region will be limited to an area somewhere away on the right side of the optimum region on the operation chart.

With a conventional homogenizer, the pressure used to homogenize ice cream mix generally is lower than that used to homogenize milk. With the knife-edged valve homogenizer, it was found that ice cream mixes could not be homogenized satisfactorily at pressures equal to or even 400 pounds higher than that which yielded satisfactory homogenization of milk. A possible reason why the cavitation homogenizer valve (knife-edged valve), when compared to the shearing homogenizer valve (poppet-type valve), did not homogenize ice cream mix as easily as it did milk, was that the ice cream mixes contained large quantities of sugar and some amount of gelatin. Sugar and gelatin consist in their chemical structures of many polar groups (molecular groups and double and triple bonds that show a strong attraction for water). Such polar groups were found to hinder cavitation by Pease and Blinks (8). The fact that adding a certain amount of air to ice cream mix raised the homogenization efficiency to a satisfactory value was a likely indication that air helped to overcome the inhibitive effect of sugar and gelatin on cavitation in ice cream mix. However, in milk the addition of a similar amount of air did not yield any significant effect on the efficiency of homogenization. A larger amount of air only reduced the homogenization efficiency. This might mean that the natural amount of dissolved gases in milk is not insufficient as far as the production of cavitation is concerned

and that the addition of air to milk only tends to cushion the hammering effect of collapsing cavities. The fact that deaerated concentrated milk was much harder to homogenize not only confirms the role of cavitation in homogenization but also may have importance in the actual production of evaporated milk. It seems that allowing milk to take up a sufficient amount of air when going from the vacuum pan to the homogenizer will insure good homogenization of the product. However, amounts more than saturation at atmospheric condition do not seem necessary.

SUMMARY

By inserting a steel cylinder between the first-stage and second-stage homogenizer blocks, it was possible to obtain samples of milk which had not gone through the second-stage valve and yet would show the influence of the backing up of pressure by the second-stage valve on the homogenization efficiency of the first-stage valve. It was found that back pressures higher than 500 psi reduced the homogenization efficiency.

Two homogenizer valves with a number of tiny holes drilled at various locations were used to show pressure variation and the progress of homogenization inside the valve clearance. Results showed that a great dip in pressure to almost atmospheric occurred near the inner edge of the valve and that homogenization was practically completed immediately beyond this location. The dip in pressure was believed to be an indication of the occurrence of cavitation.

A homogenizer valve with the valve seat narrowed down to a knife edge homogenized both milk and concentrated milk satisfactorily at a pump pressure of 800 to 1,000 psi and a back pressure of 50 to 150 psi. Ice cream mix was not homogenized satisfactorily at similar pressures unless air at a saturation pressure of 75 psi was introduced into the mix. The air was believed to help overcome the inhibitive effect on cavitation of sugar and gelatin which were present in the ice cream mix.

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PEOPLE and EVENTS
in the Dairy Science World

With this issue of the JOURNAL is inaugurated a new department, which has been named by the Journal Management Committee "People and Events." The purpose of this department is to publicize important happenings in the industry, such as transfers and promotions, new developments in laboratory, plant, and dairy farm equipment, and important trends. The success of this venture will depend largely upon the extent to which this type of information is made available to the editor. All members are urged to cooperate by sending to the editor's office by the first of each month all personal and industry news that they think worthy of consideration.

PROFESSOR H. B. HENDERSON, our president, has sent the following message to the members of the Association:

H. B. Henderson

"This is perhaps the first time in the history of the American Dairy Science Association that your president has had an opportunity to greet you through the medium of the Journal of Dairy Science, except through publication of the address delivered at the time of the annual meeting. I am sure this new feature, 'People and Events', will prove beneficial to those who read the Journal. I hope you will assist the editor by sending him items for use in this section.

"At this time I wish to express our gratitude to the retiring editor, DR. F. E. NELSON, Iowa State College, for the contribution he has made to the Journal during the past six years. It has been a difficult undertaking, and he has made many personal sacrifices in order to render this service. I know that each Journal subscriber and member of the Association would want me to thank him for a job well done.

"Furthermore, I know you will join me in welcoming the new editor, DR. PAUL H. TRACY, University of Illinois. We are, indeed, most grateful to Dr. Tracy for his willingness to assume the responsibility of editing the Journal. He is exceptionally well qualified to perform these duties, and we wish him every success.

"As we face the new year together, I hope each of us will determine to do his part in strengthening the Association so that it may be of even more value to the dairy industry. I have already written a letter to the members expressing the hope that our membership may be doubled in 1953. This is not wishful thinking. I firmly believe it is possible for each of you to secure one additional member. With your cooperation we can make 1953 a historic year for the American Dairy Science Association."

Many Retired Faculty People Now Floridians

A number of our older members who have retired from active duty are now living in one of the fastest growing dairy sections of the country—Florida. DR. H. B. ELLENBERGER, former head of the Dairy Department at Vermont, is living in Orlando. Also at Orlando is DR. E. G. HASTINGS, for many years head of the Bacteriology Department at Wisconsin, who is operating a citrus grove. DR. and MRS. F. W. TANNER have a permanent home at Winter Park. Dr. Tanner was head of the Department of Bacteriology at the University of Illinois for many years. DR. M. J. PRUCHA, Professor Emeritus of Dairy Bacteriology at the University of Illinois, and MRS. PRUCHA are starting the second year in their home at New Smyrna Beach. DR. and MRS. B. W. HAMMER are living in Sarasota, the winter headquarters of Ringling Brothers' circus. Dr. Hammer was formerly in charge of the Dairy Bacteriology Section at Ames. MR. and MRS. C. H. PARSONS have a home in Tampa. For many years Mr. Parsons was in charge of dairy research for Swift and Company in Chicago and was an active supporter of our Association. There are no doubt other retired members of the A.D.S.A. who prefer the tropical weather of Florida to the ice and snow of the north.

A.D.S.A. Booth Proves Popular at Dairy Industry Exposition

Our officers are to be commended for their foresight in arranging for an A.D.S.A. hospitality booth at the Dairy Industry Exposition at Navy Pier in Chicago, September 22 to 27. Through the cooperation of T. J. KUHLMAN of the Bowman Dairy Company, local dairies supplied hostesses to meet visitors. Space was donated by D.I.S.A. Twenty-seven thousand people are reported to have attended the exposition.

To Formulate Program for Procuring Test Animals

According to DR. W. E. KRAUSS, Director of the Ohio Experiment Station and representative to the National Dairy Council, a committee has been appointed within the Division of Biology

and Agriculture to organize a systematic program in the broad field of procurement and supply of animals for biological and medical research, assaying, testing, and related uses. Among the problems needing coordination in this field are: (a) establishment of minimum standards of acceptability and maximum limits of tolerance, (b) stimulation of production of genetically defined strains, (c) maintenance of existing strains, (d) search for new and useful mutations, (e) creation of a central registry, (f) establishment of an exchange service, and (g) improvement in mechanics of rearing, shipping, and protecting animal stocks.

New Product a Threat to Dairy Industry

It appears that a new product has been added to the controversial list of substitute dairy products. "Filled ice cream" is now being manufactured and sold in four states—Texas, Oklahoma, Missouri, and Illinois. In the November 3 issue of Food Field Reporter, THEODORE G. MONTAGUE, president of The Borden Company, states, "In the long run this problem will be solved by the court of final decision—the American consumer." If this is true, dairy products offered to the public must be of consistently high quality and must be sold at reasonable prices if they are to compete with the substitute products.

This is a challenge to our dairy scientists and leaders. According to PROFESSOR H. F. DEGRAFF of Cornell University, 53 cents of the consumer's food dollar went to farmers and 47 cents to food handling and distribution services in 1946. In 1952, 48 cents went to the farmer and 52 cents for processing and marketing functions. Will an acceptable sterile milk and the use of vending machines be a partial answer to rising distribution costs in the dairy industry?

Agricultural Research Institute Formed

DR. ROY C. NEWTON, Swift and Company vice-president in charge of research, has been elected president of the newly organized Agricultural Research Institute. B. S. CLARK of the American Can Company is secretary. On the governing board for class A members, who are composed of food manufacturers and marketers, is H. E. O. HEINEMAN, Pet Milk Company. The Institute held its organization meeting in Washington, October 16.

Creamery Package Announces Staff Changes

JOHN JOHNS was appointed sales manager of the St. Louis branch of the Creamery Package Company, as of November 1, 1952. DWIGHT ANDERSON was recently made manager of the Minneapolis branch. ROBERT RENNEBOHM has been added to the Chicago sales branch. Robert is a graduate in Dairy Industry from the University of Wisconsin, where he was captain of the 1947 football team. R. J. WADE has been made acting export manager to take the place of OSCAR SELANDER, who died December 5, at the age of 54 years.

Shortage of Students Presents Problem

Our profession is faced with a shortage of both undergraduate and graduate students. This may be explained on the basis of several factors. The military draft and the present attraction offered in other fields, such as commerce, medicine, law, and engineering, have had their influence. Possibly it is time that we re-evaluate our curricula at both the undergraduate and graduate level. It also might be well to ask industry to study the problem. A more definite indoctrination and promotion program for college graduates entering industry might attract more and better students to the dairy courses.

In a recent Management Institute meeting held at the University of Illinois, EARLE E. LANGELAND, vice-president of The American Maize Products Company, predicted that it will become increasingly difficult to secure qualified men to run our highly complicated industries. He thinks business should take more interest in education and supply financial aid to talented students who lack the means to educate themselves. He believes that if business does not do this, the Government will do so. Possibly the dairy industry should give some thought to this approach to the problem of getting competent young men to enter our field.

M. H. Alexander Goes to India

A number of our university scientists are going to foreign countries on Point Four appointments. PROFESSOR M. H. ALEXANDER of the Dairy Science Department of the University of Illinois left the Urbana campus on November 8 for the Allahabad Institute of Agriculture in India. His main project will be a feeding and breeding program for dairy cattle. When properly carried out, these assignments to foreign countries should do much to promote democratic ideals throughout the world.

New Research Directors Announced

DR. PHILIP K. BATES has been named general manager of research for the Carnation Company. The new research center for this company is to be located at Van Nuys, California. Dr. Bates was formerly president of Riker Laboratories, Inc., a subsidiary of the Brazil Drug Company. He is a graduate of M.I.T.

DR. LEWIS H. ROGERS has been appointed head of the Division of Analytical Chemistry of the National Dairy Research Laboratories, Inc., Oakdale, New York. For the past several years Dr. Rogers has been in charge of an analytical research group in a division of Union Carbide and Carbon Corporation, operating at Oak Ridge, Tennessee, under the auspices of the Atomic Energy Commission. Dr. Rogers received his B. S. and M. S. degrees from the

University of Florida in 1932 and 1934, respectively, and a doctor's degree from Cornell in 1941.

Michigan State Holds First Engineering Conference

The first annual dairy engineering conference to be held at Michigan State College has been announced for February 25 and 26. Discussions will be held on the engineering features of dairy plant operations and equipment designs.

Danish Expert Studies Our Packaging Methods

AAGE H. PEDERSON, Research Director in the State Experimental Dairy of Hillerød, Denmark, visited experiment stations and commercial organizations in Illinois, Wisconsin, and Minnesota during November. He has been studying our methods of packaging cheese and butter for retail distribution. Director Pederson believes it necessary that the cheese industry of Denmark improve the form, appearance, and keeping properties of cheese that is to be exported to the United States.

Cherry-Burrell Adds Farm Tank Milk Coolers

The success of the California system of cooling and storing milk in tanks at the farm has led to its rapid introduction to other parts of the country. A number of companies are now making this equipment. The Cherry-Burrell Corporation has announced that this company is now building two types of tanks for use on dairy farms. One type, which ranges in size from 100 to 600 gallons capacity, is shipped as a complete package unit. An ice bank makes possible storage of refrigeration, a desirable feature in case of a power failure. The milk is either poured into a strainer on top of the cooler or piped directly from the milking parlor and then is distributed over a corrugated cooler surface. It is kept cold by means of refrigeration coils in the bottom of the tank.

The Cherry-Burrell Corporation has also developed a conventional refrigerated storage vat in sizes from 100 to 1000 gallons capacity. Refrigeration is supplied by parallel channels welded to the outside bottom of the stainless steel tank lining. Rapid cooling is aided by the use of a slowly moving twin paddle agitator. The temperature of the milk is thermostatically controlled.

The customary procedure when using milk storage vats is to measure the volume of milk in the tank by means of a calibrated stainless steel rod. This is done by the milk hauler prior to mixing and sampling. The milk is pumped from the storage tank to the pick-up tank truck. Much of the milk handled in this manner on California dairy farms reaches the milk plant with a count of 1000 to 2000 bacteria per ml. The system also brings about a savings in handling and hauling costs and is an economic advantage to both the farmer and the processor.

Western Regional Officers Announced

New officers for the western division of the A.D.S.A. are as follows: president, D. L. FOURT, head of Department of Dairy Industry, University of Idaho; vice-president, GEORGE DRUMM, Department of Dairy Husbandry, California Polytechnic Institute; secretary-treasurer, LLOYD R. HUNSAKER, Department of Dairy Husbandry, Utah State College.

New Bulletin and Film Tells Story of Bulk Cooling

A new bulletin containing detailed information about bulk coolers and bulk cooling methods will be published by Mojonnier Brothers Company at an early date. The same firm is also preparing a movie showing bulk cooling installations and pickup routes in several eastern states. When completed, both the bulletin and the movie will be available for distribution and for showing at group meetings, short courses, and other gatherings.

Tri-Process Bowl Will Lower Labor Costs

Research engineers for the De Laval Separator Company have developed for the small and medium sized dairy plants a new Tri-Process bowl. This bowl, with very slight adjustments, will standardize, separate, and clarify milk, thereby saving many hours of plant labor. We need many more such developments in order to reduce labor costs in dairy plants.

Research Results Should Be Used Wisely

In the annual report issued recently by the Carnegie Foundation president OLIVER C. CARMICHAEL states that research workers often confuse truth with a collection of undigested facts. Dr. Carmichael fears that higher learning has sometimes concerned itself too much with the "cult of objectivity," which tends to develop men without definite goals. He refers to scientific Germany under Hitler as an example of such philosophy. He further states, "Pursuit of truth is undoubtedly the highest function of the university, but that is not synonymous with scientific research. It refers to search for reality, for meaning, for ultimate answers . . ." Those responsible for the direction of research in the dairy industry might well profit from these words of warning. The use made of facts brought forth by research is more important than the mere gaining of those facts. The future of society will depend largely on how well scientists of today and tomorrow use the knowledge gained through present day research.

Welcome . . .

TO THE NEW EDITOR OF THE JOURNAL OF DAIRY SCIENCE

In August, 1952, the Executive Board of the American Dairy Science Association elected Dr. Paul H. Tracy editor of the Journal of Dairy Science. The January, 1953, number of the Journal marks the beginning of his service. Dr. Tracy is the fifth editor since the Journal was founded in 1918.

Dr. Tracy has served this Association for many years in almost every elective office it has to offer. He understands the problems of the Association and knows thoroughly the responsibilities which he assumes at this time.

He is technically qualified to serve the Association in this capacity. He has contributed, as author or coauthor, over 200 papers to the scientific and technical literature during more than 30 years of academic work and scientific research. In 1946 he was given the Borden award for outstanding research in Dairy Manufactures. His publications are well known in the fields of market milk, ice cream, condensed milk, and dried milk. These papers have added to our knowledge of methods of processing milk and its products, oxidized flavors, physical properties of ice cream, ice cream mix ingredients, and the keeping properties of dried milk. He is co-author of the book "The Ice Cream Industry." Dr. Tracy's broad experience in scientific research and in writing, speaking, teaching, and consulting qualify him eminently for his new work as editor of the Journal of Dairy Science.

In 1930 he helped organize in Chicago this country's first Dairy Technology Society and has served as its secretary ever since. This activity has given him an unusual opportunity to become acquainted with the needs of dairy technologists, business men, and industrial research workers.

From 1920 until the present time Dr. Tracy has been a member of the staff of the University of Illinois. As a teacher and leader he has an enviable reputation.

The members of the Journal Management Committee believe that the Association is fortunate in this choice of editor. Dr. Tracy can be assured of the sincere cooperation of the officers and the members of the Association.

We wish for him many successful and pleasant years as editor of the Journal of Dairy Science.

J. Hoffman Erb
Ralph E. Hodgson
Walter V. Price, Chairman
Journal Management Committee
American Dairy Science Association

ASSOCIATION ANNOUNCEMENT

TO THE MEMBERS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION—WISCONSIN WELCOMES YOU!

The University of Wisconsin is honored, after 25 years, to welcome you again to its campus. We are happy indeed that you have chosen to hold your annual meeting this year in Wisconsin. We are looking forward with pleasant anticipation to June 22-24, 1953.

It is our wish that your visit to Wisconsin will be productive, profitable, and pleasant. The 48th annual meeting of the American Dairy Science Association will provide an opportunity to:

—discuss the many problems confronting the dairy industry,
—share with each other the latest scientific information, and
—formulate plans for advancement.

We suggest that you allow enough time for you to visit our dairy farms, dairy plants, and many other places of interest to dairymen. Wisconsin is particularly beautiful in June, and we hope it will be possible while you are here to enjoy some of our many recreational facilities.

We extend to you a most cordial welcome.

Yours sincerely,

E. B. FRED
President of the University of Wisconsin

PAPERS FOR THE 1953 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The 48th annual meeting of the American Dairy Science Association will be held June 22-24, 1953, on the campus of the University of Wisconsin at Madison, Wisconsin. All members wishing to present papers should submit the titles of their papers accompanied by two copies of an abstract of not more than 200 words not later than March 1 to the Chairman of their Section and an additional copy each to the Vice-Chairman and Secretary of their Section. Terminology used in the abstracts should be understandable by the general reader as well as the specialist, according to a resolution adopted in 1951. Titles and abstracts must be received promptly, since abstracts of the final program must be made available in printed form for the annual meeting. It may be necessary this year to read some papers by title only, if it is found impossible, because of time limitations, to present them in their entirety on the section program. The respective section officers are as follows:

Extension Section

Chairman: Ivan E. Parkin, Department of Dairy Husbandry, Pennsylvania State College, State College, Pa.

Vice-Chairman: Stanley Gaunt, Department of Dairy Industry, University of Massachusetts, Amherst, Mass.

Secretary: E. T. Itschner, Department of Dairy Husbandry, University of Missouri, Columbia, Mo.

Manufacturing Section

Chairman: O. F. Garrett, M. & R. Dietetic Laboratories, Inc., Columbus 16, Ohio.

Vice-Chairman: A. J. Morris, Department of Dairy Husbandry and Manufacturing, Utah State Agricultural College, Logan, Utah.

Secretary: G. H. Hartman, White House Milk Company, 102 Revere Drive, Manitowoc, Wis.

Production Section

Chairman: George Hyatt, Jr., Extension Dairy Specialist, University of North Carolina, State College Station, Raleigh, N. C.

Vice-Chairman: Philip L. Kelly, Department of Dairy Husbandry, University of Nebraska, Lincoln, Neb.

Secretary: R. E. Erb, Department of Dairy Science, State College of Washington, Pullman, Wash.

The general program committee believes that no person should present more than two papers, thereby making possible active participation by more members. No title will be considered that is unaccompanied by a suitable abstract. It is desired that more contributions be submitted by senior staff members and from the laboratories of industry.

Because of problems in slide projection, it is desirable for speakers to distribute mimeographed copies of pertinent data, together with a brief summary of the paper.

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Nomenclature and Methodology of Milk Proteins

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Standard Bacterial Plate Count of Milk—Temperatures of Incubation

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Standardization of Dairy Alkali Tests and Methods of Reporting Results

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Standardization of the Babcock Test

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Uniform Procedures for Making Acidity Tests of Fluid Dairy Products

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Soft Curd Milk

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ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

1. The effect of added manganese in feed on various mineral components of cattle blood. P. FAIN, J. DENNIS and F. G. HARBAUGH, Texas Tech., Lubbock. Am. J. Vet. Research, **13**, 48: 348-350. July, 1952.

Since "wheat poisoning" was found when animals were receiving herbage averaging 77 p.p.m. Mn, which is more than twice the Mn level of normal winter feeds, attempts were made to produce the symptoms of the disease by adding Mn to the ration of cows to furnish 75, 100, 150, and 200 p.p.m. No significant change occurred at any level of Mn feeding in blood glucose, Ca, Fe, or K. A slight decrease in blood Mg occurred at 100 p.p.m. Mn, but it was not evident at higher levels of Mn feeding, so it was assumed to be of little significance.

E. W. Swanson

2. White heifer disease in a herd of dairy shorthorns. J. M. RENDEL, Animal Breeding and Genetics Research Org., Edinburgh. J. Genetics, **51**: 89-94. 1952.

The incidence of white colored Shorthorns, all sired by six of seven bulls studied, in a large herd was compared to herd book data and theoretically calculated expectations. The a priori assumption that white heifers from white dams would have more chances to be fertile than those from roan dams was borne out by a sample from the population in general but not from the herd under consideration.

That white zygotes are more inviable, or unregistered for other reasons, than others is suggested by the deficit of 32 percent among the whites in the mating of heterzygote to heterzygote. Both white and roan cows were found to milk less than red cows. Nevertheless, after registration roans were favored by breeders.

L. O. Gilmore

3. Studies on repeated vaccination of cattle with Brucella abortus strain 19. III. The response of vaccinated and revaccinated cattle to conjunctival exposure with a virulent strain of Brucella abortus during the third gestation period. D. T. BERMAN, B. A. BEACH, and M. R. IRWIN, Wis. Agr. Expt. Sta., Madison. Am. J. Vet. Research **13**, 48: 351-358. 1952.

Three groups of 21 heifers were vaccinated, respectively, at 8 mo., 8 mo. and again at 14 mo., and at 8 mo. and again at 20 mo. All were bred for 1st pregnancy at 15-18 mo. After the 1st pregnancy, a group of non-vaccinated yearling heifers was added to the herd. During the 3rd pregnancy of the experimental herd, all animals were exposed to *Brucella* via the conjunctival sac. Following exposure, 19 of the 22 control cows became infected. Three of the 14 once-vaccinated heifers demonstrated infection; 5 of the 18 vaccinated at 8 and 14 mo. became infected; and 4 of the 16 vaccinated at 8 and 20 mo. became infected. No increased resistance could be ascribed to the repeated vaccinations. The twice-vaccinated groups developed higher blood titers after exposure than did the once-vaccinated group. In most cases these titers exceeded 1:100, even though no sign of infection was present.

E. W. Swanson

BOOK REVIEW

4. Standard methods for the examination of dairy products. Proposed changes for tenth edition. Joint Editorial Committee. Am. J. Pub. Health, **42**, 9: 1131-1134. 1952.

A "digest" is presented of the major changes suggested for the tenth edition of Standard Methods for the Examination of Dairy Products. These include the following: Revision of the directions for sampling milk and cream, for preparing dilutions of powdered milk, the application of the sediment test, and substitution of the swab method for the shaking and/or rotating method in checking the sanitation of milk cans. The plate contact method for determining surface contamination is to be omitted. The present agar used in plating milk will be replaced by milk-free plating media. Also buffered distilled water will be required for water blanks.

The use of a metal syringe to transfer the 0.01 ml. of milk used in the direct microscopic count will be introduced as will the use of micro slides with delineated, round, 1 sq. cm. areas over which the 0.01 ml. of milk or cream is spread. Three staining procedures will be permitted; acid and water free stain, aniline oil-methylene blue stain, and polychrome methylene blue stain.

Directions for the reduction type test will be revised and the dye content of the methylene blue thiocyanate and resazurin tablets will be required on the label. Application of and directions for the coliform test are to be clarified as well as the method for determination of thermoduric bacteria. The ring test for detecting brucellosis infected milk is to be included.

The AOAC (Sanders-Sager) and the N.Y. State Dept. of Health (Gilcreas-Davis) method for determining residual phosphatase in milk or other dairy products will become official.

There are also many other less important changes proposed. D. D. Deane

BUTTER

O. F. HUNZIKER, SECTION EDITOR

5. Method of separating butterfat from globules thereof. S. PATTON and C. M. STINE (assignors to the Pennsylvania Research Corp.). U. S. Patent 2,617,731. 8 claims. Nov. 11, 1952. Official Gas. U. S. Pat. Office, **664**, 2: 596. 1952.

A method for manufacturing butter oil which consists of breaking the fat emulsion of cream by the addition of an organic surface tension lowering agent and heating to at least 120° F. to melt and cause the fat globules to coalesce. R. Whitaker

6. Process for treatment of cream and milk. C. A. S. THORSTENSSON-RYDBERG (assignor to J. R. Andersson & Co.). U. S. Patent 2,617,732. 1 claim. Nov. 11, 1952. Official Gaz. U. S. Pat. Office, **664**, 2: 596. 1952.

A process for producing butter in a churn which rotates about an axis. Provision is made for changing the inclination of the axis from the vertical. An inclination of 5°-10° is used when the ripened cream is cooled, 45°-90° during churning, 90°-180° when draining the buttermilk and wash water, and 45°-90° when working the butter. R. Whitaker

7. Vertical feed butter dispenser. R. R. PERKINS. U. S. Patent 2,616,173. 7 claims. Nov. 4, 1952. Official Gaz. U. S. Pat. Office, **664**, 1: 91. 1952.

Individual servings of butter are sliced, as needed, from sticks of butter stacked in a refrigerated housing. R. Whitaker

8. Bekaempelse af mug på drittelsmør (Control of mold on butter in casks). State Expt. Sta. Creamery, Hillerød, Denmark. Report No. **74**. June, 1952.

Leaky butter more easily became moldy than butter that had been worked dry in the churn. Unsalted butter became moldy more readily than butter that contained 1% salt. Unparaffined dry casks were used. In general, it was found that dry parchment paper for lining the casks was more satisfactory than paper that had been treated with brine. To prevent mold infection of parchment by brine, the daily boiling of concentrated brine in which the parchment is immersed was an effective method. The casks of butter should be stored in a dry, properly refrigerated room in such a manner that the wood does not absorb moisture from the floor.

Growth of mold is rapid when the temperature and humidity are favorable. It was pointed out in the introduction to the report that mold mycelia generally die when the temperature is a few degrees below the freezing point of water. Spores, however, may resist a temperature of 110° C. Penicillium may grow in a refrigerator at 10° C., where the humidity is high. It was found that when casks of butter were stored in a refrigerator with a humidity of 80-84%, there was mold on all lots of butter irrespective of the method that was used in treating the casks and parchment paper. A humidity of 60% was found satisfactory. G. H. Wilster

9. Fortsatte forsøg med tempereringsmetoden (Continued experiments with the method of tempering butter). (Earlier results were published in 1939.) State Expt. Sta. Creamery, Hillerød, Denmark. Report No. **75**. June, 1952.

The principle of the method is to sprinkle the surface of a stainless steel churn with water at 90-108° F. after the completion of the working process until the butter acquires a semifluid consistency. The butter is then forced out of the churn by air under pressure inside the churn. The temperature of the butter when forced out was 68-70° F. during the summer and 72-74° F. during the winter. The butter had an undesirable consistency at the temperature at which it was generally consumed. At about 65° F., summer butter with an iodine value of about 40 was considerably softer than butter manufactured in the regular manner. At 59° F. and below, winter butter with an iodine value of about 30 was firmer than regular made butter.

In the later experiments the butter was removed and printed direct from the churn. A vacuum measuring apparatus was used. The temperature of the butter was adjusted to 63° F. during the summer and 66° F. during the winter. When these temperatures were used there was no unfavorable effect on the consistency of the butter. These temperatures may be too low to allow for the rapid removal of butter in the creameries. G. H. Wilster

10. Method of modifying butter. C. H. BETTMAN. U. S. Patent 2,612,454. 2 claims. Sept. 30, 1952. Official Gaz. U. S. Pat. Office, **662**, 5: 1421. 1952.

Butter is heated with a small amount of salt to about the boiling point, then cooled and allowed to stratify. The butter layer is recovered by removing it from the cheese-like layer and the layer of milk-like material. R. Whitaker

CONDENSED AND DRIED MILKS: BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

11. Navy interests in concentrated milk. M. W. CLAY, Navy Procurement Officer, Chicago. Milk Dealer **42**, 1: 56-58. 1952.

The Navy has two interests in concentrated milk. First, because of its keeping qualities it is a practical source of milk in areas where local milk is scarce and expensive. Second, in areas where local milk cannot be obtained, concentrated milk provides an invaluable means of building the morale of service men. It will keep in good condition for at least 2 wk. and has been reported still fresh and palatable after 5 wk. The Navy is buying some frozen concentrated milk for use in the Pacific. This milk keeps longer than the fresh concentrated milk but does not remix so well. It is also using a considerable amount of fresh concentrated milk on vessels leaving the U. S. for extended cruises. The milk used is a 3-to-1 concentrate, as experiments with a 4-to-1 concentrate were not successful. C. J. Babcock

12. Method for freezing and drying liquids and semisolids. T. R. FOLSOM. U. S. Patent 2,616,604. 4 claims. Nov. 4, 1952. Official Gaz. U. S. Pat. Office, **664**, 1: 213. 1952.

A method is described for continuously freeze-drying materials such as milk, in which the liquid is atomized into the high vacuum to form frozen porous particles, which, when dry, yield a very readily soluble dispersible powder. R. Whitaker

13. Can piercing device. W. E. SHANDS. U. S. Patent 2,614,319. 6 claims. Oct. 21, 1952. Official Gaz. U. S. Pat. Office, **663**, 3: 663. 1952.

An apparatus resembling pliers in form, one jaw of which pierces two holes in the lid of cans containing evaporated milk and other liquids, and the other jaw causes a deformation of the top edge of the can to form a pouring spout below one of the pierced holes. R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

14. Die Bedeutung der Hemmungserscheinungen zwischen Strep. lactis und Strep. cremoris für die Neuzüchtung von Säureweckern (The importance of inhibition between Strep. lactis and Strep. cremoris in preparing new starters). English summary. G. Wilharm and E. Cibilis. Milchwissenschaft, **6**, 2: 51-53; **6**, 3: 87-90. 1951.

A group of 68 single-strain lactic cultures was studied with respect to the production of growth-inhibiting substances. The group tested consisted of 8 strains of *Streptococcus cremoris* and 15 strains of *S. lactis*, all of which were isolates from 5 different multi-strain starters. An additional 45 strains of *S. lactis* were isolates from 18 samples of raw milk.

None of the cell-free filtrates from growth cultures of *S. cremoris* when added to growth media showed an inhibiting effect upon the single strain cultures of *S. cremoris* or *S. lactis*. Some of the cell-free filtrates from growth cultures of *S. lactis*, however, inhibited the growth of certain strains of *S. lactis* and *S. cremoris*.

Growth inhibition of certain single strain cultures was observed also when N/10 lactic acid was added to the growth medium in increasing quantities, namely from 0.1-1.0 ml./5 ml. yeast-dextrose bouillion. Inhibition was most pronounced with strains of *S. cremoris* followed by starter isolates of *S. lactis*. Least inhibition resulted with *S. lactis* strains, isolates from raw mil. I. Peters

15. Recherches sur les intoxications dues aux fromages (Investigations on poisonings resulting from cheese). C. AGENJO. Lait, **32**, 369-374. July-Aug., 1952.

Eight years of research on cases of food poisoning traceable to cheese established *E. coli* as an important agent. The ability of a strain to produce intoxication was strongly correlated with a high degree of pathogenic virulence toward experimental animals. Although considerable variation in heat resistance of *E. coli* strains was observed, cheese made from milk contaminated with various strains showed no evidence of *E. coli* when the milk was adequately pasteurized (65° C. for 30 min.) following contamination. S. Patton

16. Hydrolysis of fats by bacteria of the Pseudomonas genus. M. L. GOLDMAN and M. M. RAYMON, Q. M. Food and Container Inst., Chicago. Food Research, **17**, 4: 326-337. 1952.

Improvements in procedures for studying bacterial metabolism of fats are suggested, especially for preparing stable emulsion media and in measuring fat cleavage. The importance of size of fat particle in the substrate was demonstrated as was the concentration of fat. The level of substrate protein was found important with organisms having proteolytic as well as lipolytic powers. The lipolytic powers of several strains and species of the *Pseudomonas* genus were studied. F. J. Doan

17. Possible identity of "lactobacillin" with hydrogen peroxide produced by Lactobacilli. D. M. WHEEATER, A. HIRSCH, and A. T. R. MATTICK. Nature, **170**: 624. 1952.

Strains of *Lactobacilli*, isolated from cheese, were found to yield zones of inhibition against *M. pyogenes aureus*. The "antibiotic"-like material, frequently referred to as "lactobacillin", was shown to be hydrogen peroxide. The presence of hydrogen peroxide is associated with living *Lactobacilli* cells. To account for the tolerance of *Lactobacilli* for hydrogen peroxide, the theory is advanced that this organism, but not *Staph. aureus*, produces catalase which destroys the peroxide. R. Whitaker

18. A comparative test with milk stains. P. H. H. GRAY, Macdonald College, Quebec. J. Milk and Food Technol., **15**, 5: 220. 1952.

The author compared four methods of staining bacteria and body cells in milk. The methods were those of Barber, Gray, Mallmann, Churchill, and the standard recommended by A.P.H.A. Gray's method gives a pink background of milk solids with blue-stained cells,

thus relieving eye strain and shows significantly higher numbers of bacteria. H. H. Weiser

19. Importance of bacteriophage as a cause of slow starters. V. N. Smith, R. B. Parker, and P. R. Elliker, Oregon Agr. Expt. Sta., Corvallis. J. Milk and Food Technol., **15**, 5: 218. 1952.

The authors concluded that bacteriophage may be a more significant factor than generally realized in restraining the normal activity of *lactic streptococcus* starters. The addition of filtrates obtained from buttermilk and cheddar, blue and cottage cheese whey caused inhibition of a selected group of single strain cultures. Bacteriophage was demonstrated to be responsible. H. H. Weiser

20. Some cultural studies with holotrich ciliate protozoa of the sheep's rumen. B. Sugden and A. E. Oxford, Rowett Research Inst., Bucksburn, Aberdeenshire. J. Gen. Microbiol., **7**, 1-2: 145-153. 1952.

Holotrich ciliates were cultured *in vitro* at 39° C. in a restricted but not absolutely anaerobic environment. The medium consisted of basal salts, centrifuged rumen liquor or grass juice, and a source of carbohydrate. Glucose and cellobiose increased the length of culture life longer than other carbohydrates tested, and galactose and cellobiose produced greatest population densities. Grass juice appeared to be the best source of nitrogenous and mineral supplements to the culture medium. J. J. Jezeski

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

21. Beiträge zur Kenntniss der Zusammensetzung der Ziegenmilch, insbesondere auch im Hinblick auf den Gehalt an verschiedenen Eiweisskörpern während einer Laktationsperiode (Contributions to the knowledge of the composition of goat's milk with emphasis on the types of proteins present during the course of a lactation period). English summary. L. Seidler. Milchwissenschaft, **6**, 2: 58-61; **5**: 170-174. 1951.

The milk of 4 goats ranging in age from 3 to 11 yr. was analyzed every 14 d. throughout a lactation period for yield/milking, titratable acidity, specific gravity, fat, T.S., ash, Fe, Ca, lactose, casein, albumin, and globulin.

Milk yields declined toward the end of the lactation period, the decline being more pronounced in the evening milking. The titratable acidity of the fresh milk was highest with the oldest animal. The sp. gravity increased some toward the end of the lactation period. The fat content, although varying considerably from milking to milking, remained rather constant throughout the whole lactation period. The T.S. usually were slightly higher in the evening milk. Ash, Fe, Ca, lactose and total protein increased toward the end of the lactation period. Casein showed greater fluctuations from milking to milking than did the total protein, the former rising some only toward the end of the lactation. Globulin showed a definite increase at the end of the lactation period and varied greatly from milking to milking, as did albumin, although the latter failed to show a clear over-all picture. I. Peters

22. Détection de préparations humanisées de lait de vache dans le lait de femme (Detection of modified cows' milk in mothers' milk). Lait, **32**: 396-399. 1952.

Several methods of detecting the presence of cows' milk in mothers' milk are compared and discussed. S. Patton

23. The formation of lactic acid in grass-water mixtures. (Abs.) A. J. G. Barnett, Univ. of Aberdeen. Biochem, J., **50**, 2: i. 1951.

A 5-1, insulated, darkened vessel equipped for the introduction of CO_2-free air or N_2, and for the taking of samples, was used to study lactic acid development in grass silage. The maximum rate of lactic acid production was during the first 8 days at a temperature of 18 to 21°. This is at variance with the view that 37° is the optimum. The addition of molasses made little difference to the acid formation or to the pH. A. O. Call

24. The branched-chain fatty acids of butterfat. 1. The isolation from butterfat of branched-chain fatty acids with special reference to the C_{17} acids. R. P. Hansen and R. B. Shorland, Dept. of Scientific and Industrial Research, Wellington, New Zealand. Biochem. J., **50**, 2: 207-210. 1951.

Branched-chain fatty acids have not been reported previously as occurring in natural fats, with the exception of dolphin oil. The isolation technique is described in some detail. It consists of a crystallization and distillation fractionation of the methyl esters following hydrogenation. The physical properties of the various fractions are given in tables. "Although data which have so far been obtained in this work are limited to the C_{17} acids, accompanying evidence indicates the presence in butterfat of a wide range of branched-chain fatty acids. Investigation of these acids is in progress." A. O. Call

25. The branched-chain fatty acids of butterfat. 2. The isolation of a multi-branched C_{20} saturated fatty acid fraction. R. P. Hansen and F. B. Shorland, Dept. of Scientific and Industrial Research, Wellington, New Zealand. Biochem. J., **50**, 3: 358-360. 1952.

Continuing the fractionation procedures mentioned in abstract **24**, the authors claim isolating "a C_{20} saturated acid fraction containing at least three and possibly four methyl groups." The fraction is extremely soluble in solvents and has the extraordinarily low melting point of −70°. A. O. Call

26. Ultraviolet spectrophotometry in detection of food product substitutes. New Applications. R. J. Morris, R. D. MacPhee, and E. L. Randall, Univ. of Nevada, Reno. Analyt. Chem. **24**, 9: 1396-1399. 1952.

Improvements were made in existing methods for the determination of the purity of certain fatty food products. Magarine could be distinguished rapidly from butter by testing the fat for conjugated tetraenoic systems using an ultraviolet spectrophotometer. Butterfat contained 0.001 to 0.004% of these systems, whereas margarine fat contained practically none. Similarly, olive oil could be distinguished from cottonseed oil. Trienoic systems were considerably more prevalent in horse fats than in pork or beef fats.

Conjugation of the double bonds of the fat or oil with alkali was induced catalytically. Certain nonconjugated unsaturated components in the fats and oils could also be estimated.

B. H. Webb

27. Identification of stabilizing agents. M. H. Ewart and R. A. Chapman, Food and Drug Laboratories, Dept. of Natl. Health and Welfare, Ottawa, Canada. Analyt. Chem. **24**, 9: 1460-1464. 1952.

A scheme is proposed for identification of stabilizing and thickening agents when they have not been mixed with other food materials. Identification of these agents in foods can be accomplished when the substances can be separated first from the other food ingredients. Identification is based on precipitation reactions with calcium, chloride, sodium and barium hydroxides and lead acetate. Reactions of the stabilizing agents with a cationic soap, ammonium sulfate, mercuric nitrate, papain and gelatin are listed. The materials studied were pectin, de-esterified pectin, algin, Irish moss, gum tragacanth, gum karaya, locust bean gum, starch, agar, gum arabic, gum ghatti, carboxymethylcellulose, methylcellulose and gelatin. B. H. Webb

28. Thioformaldehyde and mercuric chloride treatment of protein filaments. G. K. Simpson and D. Traill (assignors to Imperial Chem. Ind., Ltd.). U. S. Patent 2,614,024. 7 claims. Oct. 14, 1952. Official Gaz. U. S. Pat. Office, **663**, 2: 510. 1952.

Thioformaldehyde hardened casein and other protein filaments are strengthened by stretching in an aqueous solution of mercuric chloride.

R. Whitaker

29. Viscosity-stable casein adhesives. T. A. White (assignor to National Starch Products Inc.). U. S. Patent 2,613,155. 6 claims. Oct. 7, 1952. Official Gaz. U. S. Pat. Office, **663**, 1: 204. 1952.

An adhesive, having a working life of several months, is composed of casein and a minimum of 50% of urea and/or ammonium thiocyanate, and 10% of an alkaline earth salt. R. Whitaker

30. Formaldehyde and uranyl salt treatment of protein filaments. G. K. Simpson and J. Brooks (assignors to Imperial Chem. Ind., Ltd.). U. S. Patent 2,614,025. 7 claims. Oct. 14, 1952. Official Gaz. U. S. Pat. Office, **663**, 2: 510. 1952.

Formaldehyde hardened casein and other protein filaments are improved by treatment with an aqueous solution of uranyl salts.

R. Whitaker

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

31. Plant floors that can take it. R. B. Seymour, Atlas Mineral Products Co., Mertztown, Pa. Food Eng., **24**, 9: 73, 74, 212, 213. 1952.

The use of 6″ x 6″ x ¾″ quarry tile in food plant floors is recommended. Such floors combine good appearance with service similar to that previously obtainable only with industrial type tile installed by bricklayer methods. The method utilizes a fast setting resinous cement spread on the floor slab. Details are given for construction by either bricklayer or tilesetter techniques. T. J. Claydon

32. Bottle washing machine. F. P. Kessler (assignor to Michael Yundt Co.). U. S. Patent 2,617,435. 3 claims. Nov. 11, 1952. Official Gaz. U. S. Pat. Office, **664**, 2: 511. 1952.

Details are given for the construction of a submerged or soaker-type of washer for milk and other types of bottles. R. Whitaker

33. Plate heat exchanger. A. H. Wakeman (assignor to The Creamery Package Mfg. Co.). U. S. Patent 2,616,671. 1 claim. Nov. 4, 1952. Official Gaz. U. S. Pat. Office, **664**, 1: 233. 1952.

A system of corrugations is described for the plates of a plate heater to increase the rate of flow between the plates. R. Whitaker

34. Cream dispenser. R. R. Perkins. U. S. Patent 2,616,607. 1 claim. Nov. 4, 1952. Official Gaz. U. S. Pat. Office, **664**, 1: 214. 1952.

Individual portions of cream are dispensed, as needed, from a refrigerated receptacle.

R. Whitaker

35. Seal for axially movable rotary plug valves. F. Hinrichs (assignor to Tri-Clover Machine Co.). U. S. Patent 2,615,672. 9 claims. Oct. 28, 1952. Official Gaz. U. S. Pat. Office, **663**, 4: 1115. 1952.

A leak sealing device for a sanitary type plug valve having the handle pivoted so that when the handle is lifted or depressed, the valve center is lifted slightly from the seat to facilitate turning the valve. R. Whitaker

36. Lift plug valve. F. Hinrichs (assignor to Tri-Clover Machine Co.). U. S. Patent 2,615,673. 3 claims. Oct. 28, 1952. Official Gaz. U. S. Pat. Office, **663**, 4: 1115. 1952.

A sanitary type plug valve, having the handle so designed that the valve core can be lifted slightly to permit easy turning.

R. Whitaker

37. Evaporator structure in refrigerating apparatus. B. J. McGovern and S. P. Skoli (assignors to Mojonnier Bros. Co.). U. S. Patent 2,617,264. 11 claims. Nov. 11, 1952. Official Gaz. U. S. Pat. Office, **664**, 2: 463. 1952.

A design for a cold wall tank refrigerated by a boiling refrigerant in conduits in the jacket of the tank. R. Whitaker

38. Vacuum regulator for milking systems. W. A. Scott (assignor to The DeLaval Separator Co.). U. S. Patent 2,613,759. 5 claims. Oct. 14, 1952. Official Gaz. U. S. Pat. Office, **663**, 2: 435. 1952.

A device is described for controlling the vacuum supplied to a milking machine. R. Whitaker

39. Automatic liquid supply regulator for milk driers. H. H. Muckala. U. S. Patent 2,612,181. 1 claim. Sept. 30, 1952. Official Gaz. U. S. Pat. Office, **662**, 5: 1341. 1952.

A float valve arrangement for maintaining the milk level between the rolls of a drum milk drier. R. Whitaker

40. Can straightener. J. L. Rickhoff and E. A. Rickhoff. U. S. Patent 2,612,204. 12 claims. Sept. 30, 1952. Official Gaz. U. S. Pat. Office, **662**, 5: 1347. 1952.

An apparatus is described for removing any dents or distortions from milk cans. R. Whitaker

41. Homogenizing device. A. G. Borck (assignor to Aktiebolaget Separator). U. S. Patent 2,612,356. 20 claims. Sept. 30, 1952. Official Gaz. U. S. Pat. Office, **662**, 5, 1393. 1952.

Milk and other liquids may be homogenized by passage through this machine, which resembles a centrifugal clarifier in design. R. Whitaker

42. Magnetically actuated milk metering device. W. H. Harstick and H. W. Hein (assignors to International Harvester Co.). U. S. Patent 2,611,269. 8 claims. Sept. 23, 1952. Official Gaz. U. S. Pat. Office, **662**, 4: 1013. 1952.

A device for metering portions of milk consisting of a two-compartment vessel sliding between two electro-magnets which control the time each compartment remains under a filler and later over an outlet. R. Whitaker

43. Weigh can dumping control. V. Schwarzkopf (assignor to The Lathrop-Paulson Co.). U. S. Patent 2,612,180. 6 claims. Sept. 30, 1952. Official Gaz. U. S. Pat. Office, **662**, 5: 1341. 1952.

Milk from a weigh can is admitted to a reservoir by a hand operated dump valve. The milk in the reservoir is removed by a milk pump activated by three electrodes in the reservoir. R. Whitaker

44. Milk can cover. R. B. Walsh and R. J. Sims. U. S. Patent 2,613,014. 1 claim. Oct. 7, 1952. Official Gaz. U. S. Pat. Office, **663**, 1: 166. 1952.

A lid for milk cans having a set of spring-attached hooks for holding the lid to the can handles. R. Whitaker

45. Combination pitcher and container for paper milk containers. R. J. Gorey. U. S. Patent 2,612,765. 1 claim. Oct. 7, 1952. Official Gaz. U. S. Pat. Office, **663**, 1: 94. 1952. R. Whitaker

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

46. The response of the rabbit uterus to instillation of semen at different phases of the estrous cycle. L. McDonald, W. G. Black, S. H. McNutt, and L. E. Casida, Wis. Agr. Expt. Sta., Madison. Am. J. Vet. Research, **13**, 48: 419-424. 1952.

The effects of 2 types of sperm suspensions upon both estrous and pseudopregnant rabbit uteri were compared. Normal semen was secured with an artificial vagina. It contained many types of bacteria. Artificial semen secured by injecting sterile water into the epididymes and withdrawing the suspension into a sterile syringe was free of bacteria. Semen was injected into the anterior portion of 1 horn of the uteri of 10-day pseudopregnant rabbits and likewise into rabbits just mated to a vasectomized buck, producing estrous conditions; 24 hours later the uteri were removed and examined grossly and histologically. Neither type of semen produced any unusual reaction in estrous rabbits. Bacteria-free semen produced no significant reaction in the pseudopregnant uteri of 4 rabbits. Normal semen containing bacteria caused local inflammation and gross pyometra in 4 pseudopregnant rabbit uteri, involving only the injected horn. The influence of hormonal balance upon uterine infection is stressed. E. W. Swanson

47. Note on the ageing of bovine embryos. R. B. Maneely, Univ. of Liverpool. Vet. Record, **64**, 35: 509-511. 1952.

Author presents in tabular form crown-rump lengths-to-age and weight-to-age relationship for bovine embryos as reported by various workers. Included in this paper are age, weight, and length relationships for 5 embryos of known age studied by the author. R. P. Niedermeier

48. Preservation of bull semen at —79° C. C. Polge and J. E. Lovelock, Natl. Inst. for Medical Research, Mill Hill, London. Vet. Record, **64**, 27: 396-397. 1952.

In view of the practical importance of freezing semen, the authors describe a simple apparatus to provide slow cooling automatically. This apparatus would be adaptable to field conditions. The ampules containing semen are placed in an aluminum can and covered with alcohol to provide an even transfer of heat. This can then is placed in a polythene beaker containing a 20% glycerol solution. When this unit is immersed in the −70° C. bath, the temperature of the 20% glycerol mixture drops slowly as water is frozen out until −15° C. is reached, then drops rapidly to −79° C. Thus, the recommended method of freezing semen can be simply followed. R. P. Niedermeier

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

49. Milk for the future and how to get it. R. E. HODGSON, Bur. Dairy Indus. Washington, D. C. Am. Dairy Prod. Rev., **14**, 9: 8, 10, 12, 14, 51. 1952.

An estimated U. S. population of 190 millions in 1975 will require 146.3 billion lb. of milk to supply the same amount per capita as in 1950 (770 lb.). This amount could be produced by the same number of cows as in 1950 with a production per cow of 6,680 lb. which is well below the 9,172 lb. average production of cows in DHIA herds. With good breeding, feeding, and management practices, such production could be obtained. Most immediate increase can be achieved through better feeding practices. Data are presented to show that an abundance of good quality roughage would afford the greatest opportunity to feed for maximum milk yields at lowest cost. Grassland farming, with more emphasis on pastures and harvested forages, is an important part of the program.

T. J. Claydon

50. Milk tank trucks are banishing the can. A. V. GEMMILL. Food Eng., **24**, 9: 143, 145, 213, 214. 1952.

The basic units of the system of bulk milk handling are the refrigerated farm tank and insulated tank truck. The cost of the farm installation may be from $1,500 to $2,500 depending on tank size. Advantages of the system tend to offset the investment.

T. J. Claydon

51. Support for milking apparatus. H. B. BABSON (assignor to Babson Bros. Co.). U. S. Patent 2,613,636. 9 claims. Oct. 14, 1952. Official Gaz. U. S. Pat. Office, **663**, 2: 399. 1952.

A support for a mechanical milking machine to be used to milk a cow standing on an elevated platform or stall. The milker is mounted on a swinging arm and the flexible suction and milk lines pass through the floor of the stall to a vacuum pump and milk collecting vessel below the stall.

R. Whitaker

52. Veterinary pen. C. B. HUTCHINGS. U. S. Patent 2,616,392. 4 claims. Nov. 4, 1952. Official Gaz. U. S. Pat. Office, **664**, 1: 153. 1952.

A pen for securing cows and other farm animals for treatment by a veterinarian.

R. Whitaker

53. Cattle feed conveying and dispensing apparatus. J. G. SPICKLER (assignor of 4/5 to Taylor E. Decker). U. S. Patent 2,615,424. 25 claims. Oct. 28, 1952. Official Gaz. U. S. Pat. Office, **663**, 4: 1043. 1952.

Silage and other feed is conveyed to cattle between two rows of stalls by an endless conveyor, but equipped with hinged buckets.

R. Whitaker

54. Suspended milker. C. A. THOMAS (assignor to Babson Bros. Co.). U. S. Patent 2,610,609. 8 claims. Sept. 16, 1952. Official Gaz. U. S. Pat. Office, **662**, 3: 744. 1952.

Details are given for the lid of the reservoir of a milker of the suspended type.

R. Whitaker

55. Milking machine teat cup assembly. J. L. DAVIS. U. S. Patent 2,612,136. 2 claims. Sept. 30, 1952. Official Gaz. U. S. Pat. Office, **662**, 5: 1329. 1952.

A design for a vacuum operated teat cup.

R. Whitaker

56. Animal fountain. R. C. LINDEMANN. U. S. Patent 2,612,138. 4 claims. Sept. 30, 1952. Official Gaz. U. S. Pat. Office, **662**, 5: 1330, 1952.

A drinking fountain for stock, connected to hot and cold water, for providing tempered water when a level is depressed by the animal's nose.

R. Whitaker

57. Control unit for milking machines. A. G. PERKINS. U. S. Patent 2,615,423. 12 claims. Oct. 28, 1952. Official Gaz. U. S. Pat. Office, **663**, 4: 1042. 1952.

A device is described for releasing milk from a vacuum-operated milking unit into a reservoir.

R. Whitaker

58. Milking structure. G. R. DUNCAN. U. S. Patent 2,617,382. 3 claims. Nov. 11, 1952. Official Gaz. U. S. Pat. Office, **664**, 2: 497. 1952.

A design for a milking stall, wherein two cows standing back to back are milked by a machine with two sets of teat cups. The milk is discharged into cans in a refrigerated tank.

R. Whitaker

59. Means for milking and handling the milk of farm animals. G. R. DUNCAN. U. S. Patent 2,617,383. 16 claims. Nov. 11, 1952. Official Gaz. U. S. Pat. Office, **664**, 2: 497. 1952.

The process is described for using the equipment mentioned in Abstract 58. R. Whitaker

60. Cow tail clamp. R. REBARCHEK. U. S. Patent 2,616,393. 1 claim. Nov. 4, 1952. Official Gaz. U. S. Pat. Office, **664**, 1: 153. 1952.

A hand clamp for holding a cow's tail.

R. Whitaker

61. Control unit for milking machines. A. G. PERKINS. U. S. Patent 2,614,530. 11 claims. Oct. 21, 1952. Official Gaz. U. S. Pat. Office, **663**, 3: 724. 1952.

The construction of a milking machine is given, having a special feature consisting of teat cups which are sealed off automatically as soon as the milk flow stops. R. Whitaker

62. Rack for milk buckets or like containers. J. L. KJELDSEN. U. S. Patent 2,614,700. 3 claims. Oct. 21, 1952. Official Gaz. U. S. Pat. Office, **663**, 3: 772. 1952.

A rack for holding clean empty milk cans in an inverted position prior to use.

R. Whitaker

63. Rotary milk can and bucket rack. D. C. ZEIS. U. S. Patent 2,612,999. 1 claim. Oct. 7,

1952. Official Gaz. U. S. Pat. Office, **663**, 1: 162. 1952.

A circular revolvable rack for holding milk cans, pails, strainers, etc., in the farm milk house. R. Whitaker

64. Container for feeding suckling calves. E. J. Hiller. U. S. Patent 2,613,642. 1 claim. Oct. 14, 1952. Official Gaz. U. S. Pat. Office, **663**, 2: 401. 1952.

A cylindrical shaped compartmented feeder for simultaneously feeding 4 suckling calves by means of nipples. R. Whitaker

65. Lid for suspended milkers. T. B. Crawford (assignor to Babson Bros. Co.). U. S. Patent 2,613,637. 6 claims. Oct. 14, 1952. Official Gaz. U. S. Pat. Office, **663**, 2: 400. 1952.

A specially designed lid for a milking machine receiving vessel suspended by a harness across the cow's back, which directs the teat cup claw directly toward the udder. R. Whitaker

66. Milking barn. R. R. Graves (assignor to The Graves-Stambaugh Corp.). U. S. Patent 2,610,608. 9 claims. Sept. 16, 1952. Official Gaz. U. S. Pat. Office, **662**, 3: 744. 1952.

A floor plan is given for a milking parlor, essentially square in shape. The cows walk around a passage on the outside of a depressed area where the operator can conveniently wash the udders and milk the cows. A series of gates control the cows' progress. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

67. Frozen confection on a stick. T. W. Turner. U. S. Patent 2,614,047. 2 claims. Oct. 14, 1952. Official Gaz. U. S. Pat. Office, **663**, 2: 516. 1952.

A frozen stick confection consisting of several layers, varying in color, etc., the concentration of the syrup in each layer being different from the adjacent layers. R. Whitaker.

68. Mixer for frozen desserts. W. C. Kelley and H. C. Phillips (assignors to Minick's Ice Cream Co., Inc.). U. S. Patent 2,611,588. 9 claims. Sept. 23, 1952. Official Gaz. U. S. Pat. Office, **662**, 4: 1105. 1952.

Ice cream is rapidly agitated by a motor driven beater in a cylindrical vessel and when softened, is withdrawn through an outlet valve directly into cups, cones, etc., for immediate consumption. R. Whitaker

69. Ice cream freezer with refrigerant control. J. W. Lofstedt (assignor to Metromatic Products Co.). U. S. Patent 2,610,478. 1 claim. Sept. 16, 1952. Official Gaz. U. S. Pat. Office, **662**, 3: 706. 1952.

A horizontal batch type ice cream freezer with the refrigerant fed in the bottom of the jacket and withdrawn from the top. The thickness of the jacket increases from the bottom to the top, and the jacket is divided into two sections, one on each side of the cylinder to insure uniformity of freezing. R. Whitaker

70. Stabilizer—emulsifier studies. H. A. Bendixen and C. W. Decker. State College of Wash., Pullman. Am. Dairy Prod. Rev., **14**, 9: 18-20, 22, 24. 1952.

Comparisons were made of the effects of several stabilizers, either alone or in combination with certain emulsifying agents, on ice cream and sherbet mixes and on the body, texture, and melting quality of ice cream and sherbet. The algin stabilizers (Dricoid and Dariloid) produced lower viscosity in mixes aged 24 hours than did gelatin. The whipping ability of the gelatin mixes was improved by mono- and diglycerides. Dricoid produced a dry appearing and slow melting ice cream. Kelcoloid, an algin derivative, which may be readily dispersed in a mix at 60° F., produced an ice cream that melted somewhat more freely than when gelatin was used. Sherbelizer, an algin derivative plus vegetable gum, permitted more rapid incorporation of air during freezing of sherbets than did pectin or Kelcoloid. Sherbilizer produced a smoother textured sherbet than did pectin or Kelcoloid. T. J. Claydon

71. Apparatus for separating frozen articles from molds. W. E. Heise. U. S. Patent 2,614,-510. 10 claims. Oct. 21, 1952. Official Gaz. U. S. Pat. Office, **663**, 3: 718. 1952.

A machine is described for defrosting molds in a brine tank operation. R. Whitaker

72. Combination Package. R. O. Tardiff (assignor to Breyer Ice Cream Co.). U. S. Patent 2,617,577. 1 claim. Nov. 11, 1952. Official Gaz. U. S. Pat. Office, **664**, 2: 552. 1952.

A box for two ice cream tarts, with an inner tray or sleeve with notches for rigidly holding the cups in a fixed position within the carton. R. Whitaker

73. Mold freezing apparatus. W. E. Heise. U. S. Patent 2,614,403. 2 claims. Oct. 21, 1952. Official Gaz. U. S. Pat. Office, **663**, 3: 686. 1952.

A brine tank for making molded frozen confections designed to maintain a uniformly low temperature around the molds. R. Whitaker

MILK AND CREAM

E. O. HERREID, SECTION EDITOR

74. Adhesives for packaging milk products. Earl C. Lenz, Paisley Products, Inc., Chicago. Milk Dealer, **42**, 1: 46-47, 90-94. Oct., 1952.

The development of resin adhesives and their relationship to the packaging problems of the dairy industry is traced. The original adhesives used in the manufacture of paper containers were: (a) Starch or dextrine adhesives, made from corn, tapioca, potato or wheat raw materials. (b) Animal base adhesives, from hides or bones of animals. (c) Casein glues, a by-product of the milk industry itself. With the exception of casein glues, they cannot be considered as water resistant adhesives. Synthetic resins came into use during World War II, and now the majority of all dairy product paper

containers are formed or sealed with modern resin adhesives, principally of vinyl polymer base. The preparation, characteristics, and requirements of these adhesives are discussed and directions are outlined for obtaining the most satisfactory results with them.

C. J. Babcock

75. Effect of grass silage on quality of milk and milk products. W. V. PRICE, Univ. of Wisconsin, Madison. Milk Dealer, **41**, 12: 124-133. Sept., 1952.

The preparation and characteristics of grass silage and its effect on quality of milk and milk products are discussed and summarized as follows: (a) Grass silage production has become an essential part of successful farming practice in Wisconsin; it seems destined to be as significant in years to come as corn silage has been in years past. (b) Improperly made grass silage of low quality has low acidity, undesirable odor and off color. Improperly made grass silage may contain large numbers of the spore-forming, gas-producing organisms capable of causing defective Swiss cheese. (c) High acidity in silage discourages the growth of the undesirable spore-forming organisms; low acid, improperly made silage favors their growth. (d) Spore-forming organisms which can cause defective Swiss cheese are commonly present in soil, manure, and some feeds. They probably are always found to some extent in the usual milk supply. (d) Spore-forming organisms in the feed pass through the alimentary tract of the cow and are present in large numbers in the manure. They cannot be secreted with the milk. (f) Sanitary methods of milk production on the ordinary farm can reduce the bacterial contamination of milk to an extremely low level. (g) Careless methods of milk production invite excessive contamination with all undesirable organisms in the vicinity of these milk handling operations. (h) Unsanitary methods may provide favorable conditions for build up of spore-forming, gas-producing organisms; such build up may occur at either the farm, the factory level or both. Once established, they are difficult to eliminate. (i) Such careless practices are unnecessary, undesirable, and illegal. Definite provisions have been established by regulations of the state of Wisconsin to prohibit and eliminate them. Grass silage of good quality can be used as well as corn silage for dairy herds if it is properly made and handled and it is fed after milking. Grass silage of inferior quality should not be used for producing milk for cheese, especially Swiss. C. J. Babcock

76. Apprøvning af A.P.V. pladepasteur, type H M med rørformet temperaturholder, A.P.V. kontrol tavle (Tests on A.P.V. short-time pasteurizer and control panel). State Expt. Sta. Creamery, Hillerød, Denmark. Report No. **76**. Dec., 1951.

The pasteurizer was obtained from the A.P.V. Company in London, England. Capacity was 6,000 l. per hr. The milk became phosphatase negative when it was heated to 72° C. Bacterial destruction was satisfactory. The flow of milk through the holder was satisfactory. The controls maintained a constant temperature of the milk, within 0.5° C. G. H. Wilster

77. Milk sanitation honor roll for 1950-52. Anonymous. Pub. Health Reports, **67**, 9: 914-916. 1952.

A total of 258 cities and counties are included on the Public Health Service "honor-roll" of safe milk communities for the period from July 1, 1950, to June 30, 1952. These communities have a rating of at least 90% compliance with the USPHS Code. The rules for inclusion on this "honor-roll" also are given.

D. D. Deane

78. Paying producers for fat and solids-not-fat in milk. Anonymous. Milk Dealer, **41**, 12: 134-140. Sept., 1952.

A condensation of the material found in Research Bull. 143 of the Agr. Expt. Sta. and Agricultural Extension Service of the Univ. of Wisconsin, 1942, is given. With changes in dairy products and demands for them, the dairy industry is becoming increasingly aware that the payment for milk from the producers, based only on the fat content, is not equitable. Several currently workable formulae for different milk markets are suggested. C. J. Babcock

79. National trend in the bulk tank system of handling farm milk. EDWARD THOM. Milk Dealer, **42**, 1: 42, 63-66. Oct., 1952.

The bulk tank system of handling farm milk is here to stay and the system is going to spread. A nationwide survey recently made by the Milk Dealer produced data from 42 of the 48 states and the District of Columbia. This survey revealed that 21 states and the District of Columbia have dairy plants with producers using the bulk tank system of handling farm milk. Ten states have at least some producers using farm tanks and six additional states have processors and producers seriously considering the adoption of the system. C. J. Babcock

80. Single or dual operation of glass and paper. Anonymous. Milk Dealer, **42**, 1: 49-54. Oct., 1952.

Survey indicates that advantages outweigh disadvantages by going 100% to one type of container. The consensus of opinion among milk dealers contacted is that 100% glass operation or 100% paper operation is cheaper than dual operation. C. J. Babcock

81. Device for carrying milk bottles. M. SADECK. U. S. Patent 2,611,639. 2 claims. Sept. 23, 1952. Official Gaz. U. S. Pat Office, **662**, 4: 1120. 1952.

A handle for carrying two milk bottles, attached to a resilient wire loop snapped around the neck of each bottle. R. Whitaker

82. Dispensing valve for liquid and gas containers. H. F. TOMASEK and F. ZIMMER (assignors to Reddi-Wip, Inc.). U. S. Patent

2,615,597. 8 claims. Oct. 28, 1952. Official Gaz. U. S. Pat. Office, **663**, 4: 1092. 1952.

A valve is described for releasing cream under gas pressure to form whipped cream.

R. Whitaker

83. Milking process. R. R. GRAVES (assignor to The Graves-Stambaugh Corp.). U. S. Patent 2,616,809. 20 claims. Nov. 4, 1952. Official Gaz. U. S. Pat. Office, **664**, 1: 273. 1952.

A process is described for handling milk, comprising the following steps: milking cows with a vacuum-operated milker, homogenizing the mixed milk without heating or cooling directly from the milking machine, and immediately pasteurizing, maintaining the milk throughout the entire process without contact with air. R. Whitaker

MILK SECRETION

V. R. SMITH, SECTION EDITOR

84. Phospholipin metabolism in the lactating mammary gland of the rabbit. (Abs.) G. A. GARTON and G. POPJAK, Natl. Inst. for Med. Research, London. Biochem. J., **50**, 3: xvi. 1952.

Rabbits were injected intravenously with ^{32}P-labelled orthophosphate. The plasma from these animals which contained ^{32}P-labelled phospholipins was then injected into a lactating rabbit. Two hours later the animal was sacrificed and the liver and mammary gland given a P fractionation treatment. Of the mammary gland phospholipins 4.3% originated from the blood phospholipins during the 2-hour period. The corresponding figure for liver was 5.2%. Radioactive phospholipins were isolated from the milk. A. O. Call

85. Aspects of fat metabolism in the ruminant with special reference to the biosynthesis of milk fat. (Abs.) S. J. FOLLEY, Univ. of Reading. Biochem. J., **51**, 3: xv. 1952.

It is postulated that milk fatty acids are of dual origin, some coming from degradation products furnished by the blood and others being synthesized in the udder from small molecules such as acetate. A. O. Call

86. The effect of thyroxine, thiouracil, and stilboestrol on the metabolism of carotene and vitamin A in lactating goats. (Abs.) R. CHANDA, H. M. CLAPHAM, and E. C. OWEN, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **50**, 2: iii. 1951.

Thyroxine increases, and thiouracil decreases, the digestibility of carotene in lactating cows. The same results have been found with goats, but the thyroxine increase is not so great in goats. On the other hand, the decrease due to thiouracil is greater in goats. A. O. Call

87. Pathways of pyruvate metabolism in the mammary gland. C. TERNER, Univ. of Reading. Biochem. J., **50**, 2: 145-154. 1951.

Lactating mammary gland tissues from rats, rabbits, and goats were incubated in various substrates in the Warburg apparatus, and the effects on respiration were measured. It is concluded that Krebs citric acid cycle is operative in the mammary gland. Pyruvate is metabolized by oxidation and also enters in synthetic reactions, with about 2 moles of pyruvate being used for synthesis for every one being oxidized. It is assumed that it is utilized in the formation of fatty acids both as a source of energy and also for fatty acid carbon.

A. O. Call

88. Cocarboxylase and protein-bound aneurin in relation to phosphatase in colostrum and milk of cows. (Abs.) R. CHANDA and E. C. OWEN, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **51**, 3: xix-xx. 1952.

It is concluded that aneurin (British name for thiamine) in the protein-bound form and also cocarboxylase (thiamine pyrophosphate) are negatively correlated with phosphatase in colostrum and in milk. The vitamin content is highest in colostrum and gradually decreases with the advance in lactation. A. O. Call

NUTRITIVE VALUE OF DAIRY PRODUCTS

ROBERT JENNESS, SECTION EDITOR

89. The effect of thyroxine and thiouracil on the composition of milk. 2. The carotene and vitamin A content. R. CHANDA and E. C. OWEN, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **51**, 3: 404-417. 1952.

Two separate experiments are reported. In the first 6 lactating Ayrshire cows were used and in the second 8 lactating goats. The experiment with cows was for 114 days, covering 7 periods of changed diet and/or thyroxine or thiouracil injections. With the goats the duration was (apparently) 53 days, and the diet was constant. Thiouracil, or thyroxine, or thyroxine and stilboestrol were given for a 2-week period near the middle of the test period. The goats' milk contained 142 I.U. Vitamin A/100 ml.—all in the ester form. Cows' milk contained 88 I.U./100 ml., but 6% of it was in the alcohol form. The goat milk contained no carotenoids, except in the colostrum. Cows on the low carotene diet gave milk with lower Vitamin A and carotene content and this was more pronounced when thyroxine was superimposed on this diet. Under these conditions the proportion of Vitamin A alcohol increased. Thiouracil had an opposite effect to thyroxine. The changes in Vitamin A and carotene in the goats' milk were similar to those occurring in the cows' milk.

A. O. Call

90. The effect of thyroxine and thiouracil on the composition of milk. 3. The concentration of the major constituents and of some of the water-soluble vitamins in cow's milk. R. CHANDA, M. L. MCNAUGHT, and E. C. OWEN, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **51**, 4: 543-552. 1952.

Five experiments, usually involving six cows in each, are reported. Two cows acted as the control group, two were given thyroxine over a 3-week period in the middle of the 9-week tests, and two were similarly given thiouracil. In general the changes noted in milk composition due to the hormones were: thyroxine decreased milk yield, fat in the milk, lactose in the milk (only slightly) and ascorbic acid (25%). Thiouracil caused the opposite effects. No changes were noted in the freezing point, Ca, Na, K, Mg, protein, creatine, or riboflavin content of the milk. The total thiamine (aneurin) content was not affected by thyroxine, although it was decreased slightly by thiouracil. Thyroxine did, however, increase the cocarboxylase (thiamine pyrophosphate) and the protein-bound thiamine, but these increases were at the expense of free thiamine. Thiouracil had an opposite effect in this thiamine partition. There was a negative correlation between the phosphatase content and the cocarboxylase as well as the protein-bound thiamine. Some discussion is devoted to the significance of these changes as they relate to milk secretion.
A. O. Call

91. Ascorbic acid in tocopherol-enriched milk. A. D. HOLMES, Mass. Agr. Expt. Sta., Amherst. Food Research, **17**, 4: 367-369. 1952.

Alpha tocopherol added to mixed herd milk in the form of an ether solution and at the rate of 0.2-2.4 mg./l. showed only a slight tendency to decrease the rate of loss of ascorbic acid when the milk was held in darkness at 50° F. for 96 hours. F. J. Doan

92. The nutritive value of milk proteins. I. Stability during sterilization and storage of evaporated milk as determined by the rat depletion method. A. Z. HODSON, Pet Milk Co., Greenville, Ill. Food Research, **17**, 2: 168-171. 1952.

No significant losses in protein nutritive value were found to occur as a result of sterilization of evaporated milk or of storage of the product for 16 months at room temperatures. Measurable losses were noted, however, in evaporated milk stored similarly for a 5-year period.
F. J. Doan

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

93. The effect of mastitis on the phosphatase and phosphorylated esters in cow's milk. (Abs.) R. CHANDA, E. C. OWEN, and P. S. WATTS, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **50**, 2: ii. 1951.

An increase in phosphatase paralleled the increase in chlorides when a quarter was affected by mastitis. The N content of the milk was increased and the P content decreased by mastitis. A. O. Call

94. The role of ammonia in ruminal digestion of protein. I. W. MCDONALD, Inst. of Animal Physiol., Cambridge. Biochem. J., **51**, 1: 86-90. 1952.

Sheep provided with rumen fistulae were used in the experiments reported. The rumen contents were strained through muslin and the ruminal fluid thus obtained was analyzed for total nitrogen, ammonia, and non-protein nitrogen. Protein nitrogen was calculated as total N minus N.P.N. When the diets were supplemented with casein or gelatin there was a marked increase in the ammonia nitrogen but this was not the case when zein was used. When starch was added to the control diet, the ammonia nitrogen level dropped. Some discussion is devoted to the possible role of the microorganisms in the digestion of protein in the rumen. A. O. Call

95. The synthesis of lysine by bacteria during incubation of rumen contents in vitro. (Abs.) M. L. MCNAUGHT, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **50**, 2: i-ii. 1951.

Rumen liquid was assayed microbiologically for lysine both before and after incubation. It was found that there was a synthesis of lysine. In one case this amounted to a 12% increase.
A. O. Call

96. The estimation of acetone bodies. C. THIN and A. ROBERTSON, Univ. of Edinburgh. Biochem. J., **51**, 2: 218-223. 1952.

As an aid in the study of bovine ketosis a method was developed for the estimation of acetone, acetoacetic acid, β-hydroxybutyric acid and isopropanol. The basis of the method is the color developed by the reaction with alkaline salicylic aldehyde. The application of the method to milk, blood, urine, and rumen liquor is described. A. O. Call

97. Methane fermentation in the rumen of cattle. W. H. BEIJER. Nature, **170**, 4327: 576. 1952.

Using a goat with a rumen fistula, it was shown that formic acid is the chief source of the relatively large amount of methane formed in the rumen. Succinic acid also produces methane, but acetic, propionic, and butyric do not. Rumen microflora, utilizing cellulose, were shown to produce both formic and succinic acid as regular metabolites. R. Whitaker

98. The capacity of bovine "stomachs." R. V. BLAMIRE, Metropolitan Cattle Mkt., Islington. Vet. Record, **64**, 34: 493-494. 1952.

Results of stomach capacity studies on 77 bovines are presented, giving weights of stomach parts and contents. The highest estimated total capacity found was 17 gal. in a cow fed heavily 3 days prior to slaughter. The author expresses the opinion that present accepted standards of bovine stomach capacities are high, and possibly a revision of these should be considered. R. P. Niedermeier

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

99. In-place cleaning of sanitary pipe lines. A. V. MOORE, A & M College of Texas, College Station. Milk Dealer, **41**, 12: 146-151. Sept., 1952.

Experiments indicate it is not necessary to dismantle steel milk lines to clean and sanitize them. The results are based on circulating rinse solutions, made up in the receiving vat, through the box-tube preheater filter and the entire line up to the holding tanks. The total length was about 180 ft. of 1.5-in. line. Rinses were conducted through a 50-ft. length of 1.5-in. tubing connecting the end of the sanitary line and receiving vat. The following sequence of rinse solutions was employed: (a) Water at 110-120° F., flushed through but not recirculated; this rinse was continued until it no longer appeared milky. (b) Thirty gal. of acid solution was made up in the receiving vat, according to the manufacturer's directions, and recirculated at 125° F. for 40 min. This solution then was discarded. (c) Thirty gal. of water at 100° F. recirculated for 5 min., then discarded. (d) Thirty gal. of alkaline solution, made up according to the manufacturer's directions, recirculated for 40 min. at 125° F., then discarded. (e) Cold water flushed through, but not recirculated for 5 min. (The system then stood until the next morning, about 17 hr. elapsing, except 1 d. additional over Saturday to Monday.) (f) Before processing began, a new filter cloth was installed, the homogenizer included in the circuit and 30 gal. of 200 p.p.m. hypochlorite solution pumped through the system. This was not recirculated but went on through the holding vats, pump, plate cooler, surge tank and bottle filler. The hypochlorite finally was discarded as some of it was "milked" out of each of the filler valves. A summary of the bacteriological quality of the homogenized-pasteurized-bottled milk shows that the cleaning in place of steel milk lines employing acid and alkaline rinses, and sanitizing them with chlorine, is a satisfactory procedure.

C. J. Babcock

100. Tips on cleaning and operating cleaned-in-place pipelines. H. P. HODES, Tri-Clover Machine Co., Kenosha, Wisconsin. Food Eng., **24**, 9: 80-82, 212, 213. 1952.

C. I. P. (cleaned-in-place) pipelines provide a number of advantages. Successful operation of the system depends on several basic rules: 1. All horizontal piping must be sloped for drainage. 2. All side lines and equipment not part of the C. I. P. circuit should be cleaned by brushing; hence provision must be made to by-pass these units. 3. An average flow velocity of not less than 5 ft/sec. should be maintained through the cross section of the largest pipe or fitting during the circulation cleaning of the system. The circulation unit is the heart of the system and the solution tank should have a capacity greater than the volume of the line being cleaned. New fittings and gaskets have been designed to facilitate operation of the C.I.P. system. Precautions and suggestions are given for installation and cleaning procedures.

T. J. Claydon

101. The determination of the bacterial content of farm equipment by the swab method. S. B. THOMAS, D. G. GRIFFITHS, E. DAVIES and N. B. BEBBINGTON. Dairy Ind., **17**, 7: 607-611. 1952.

The swab technique of the British Ministry of Agriculture was found to give a more dependable evaluation of the sanitary condition of farm milking equipment than the rinse method. Faster transportation of swabs to the laboratory and incubation of plates for 4 days at 30°, rather than the prescribed 2 days at 37°, were shown to be desirable changes in the technique.

R. Whitaker

102. New developments in detergent-sanitizers. N. E. LAZARUS, Lazarus Laboratories, Inc., Brooklyn, N. Y. Milk Dealer, **42**, 1: 45, 68-71. Oct., 1952.

A brief review of sanitation as it has developed in the dairy industry with special reference to the germicidal aspect which includes hot water, steam, hypochlorites, quaternaries, and iodophors. The major limitation to use of hypochlorites for dairy farm and plant sanitation are offensive odors and bad taste, reduction of efficiency by oxidizable contaminants, skin sensitivity, and lack of penetration. They are inhibited in their action on various types of organisms but are effective with Gram-negative bacteria such as coliform. The favorable factors of the quaternaries are good penetration, stability, non-toxic and non-irritant action in use dilutions, freedom from offensive odors, and volatization. The hindrance factors are reduction of germicidal activities in hard waters, inability to destroy pseudomonas bacteria, and the lack of a residual test. In a combination of iodine and carrier, which is called an iodophor, the germicidal activity of iodine is enhanced to a marked degree and the vapor pressure is reduced almost to the vanishing point—primary requisite in iodine stability. The characteristic odor of iodine is reduced to a minimum, permanent stain is eliminated, and the intense stinging sensation which accompanies the use of tincture of iodine is not found in iodophors. They can be diluted for use to a greater degree and are not affected by water hardness. After thorough laboratory evaluations an iodophor compound was prepared and tested in the field. The results of the field test indicate that the iodophor compound is an advanced step in dairy farm sanitation even under the most severe conditions.

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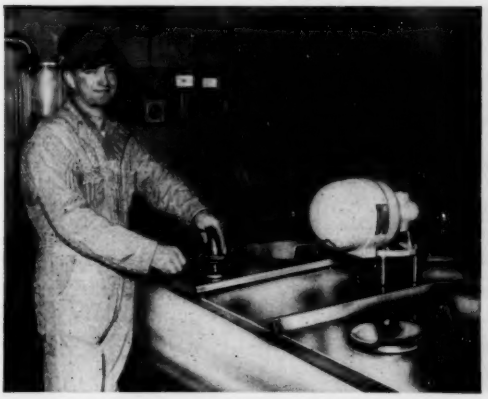

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